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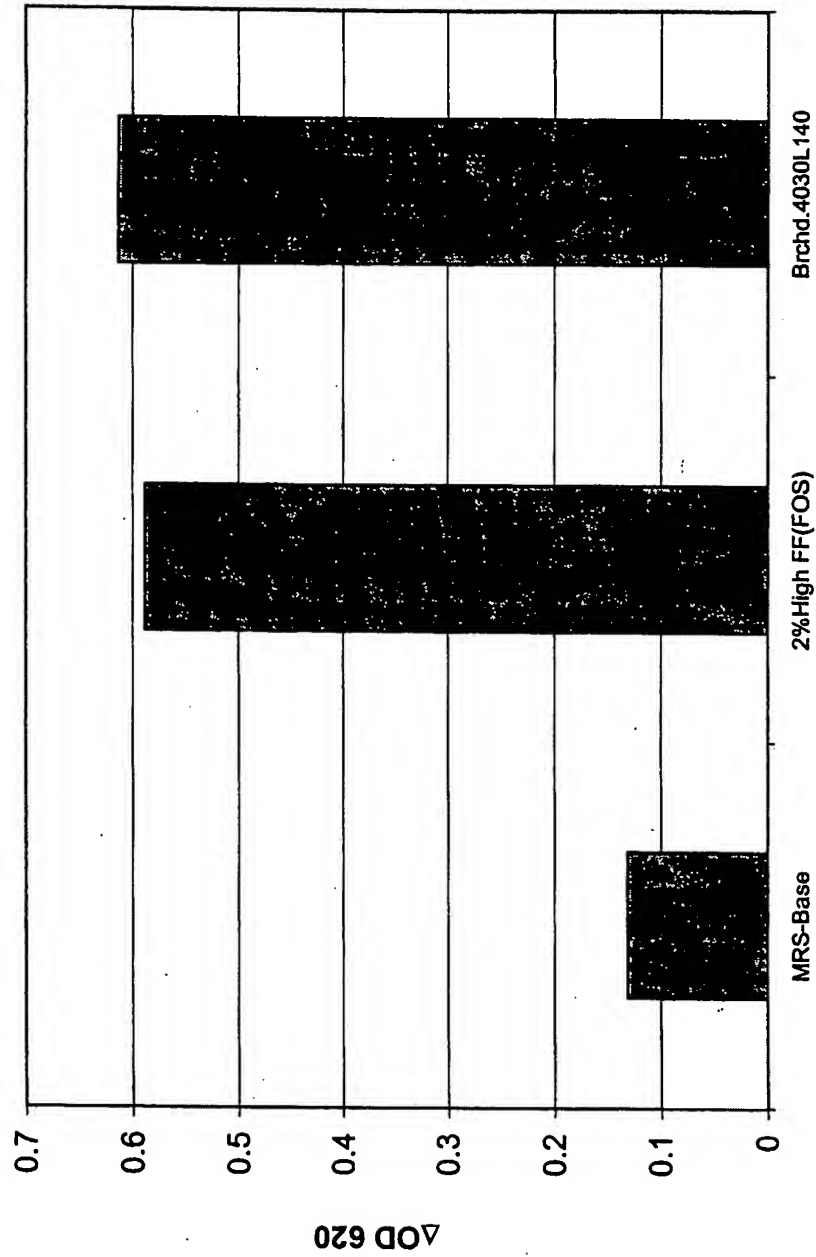
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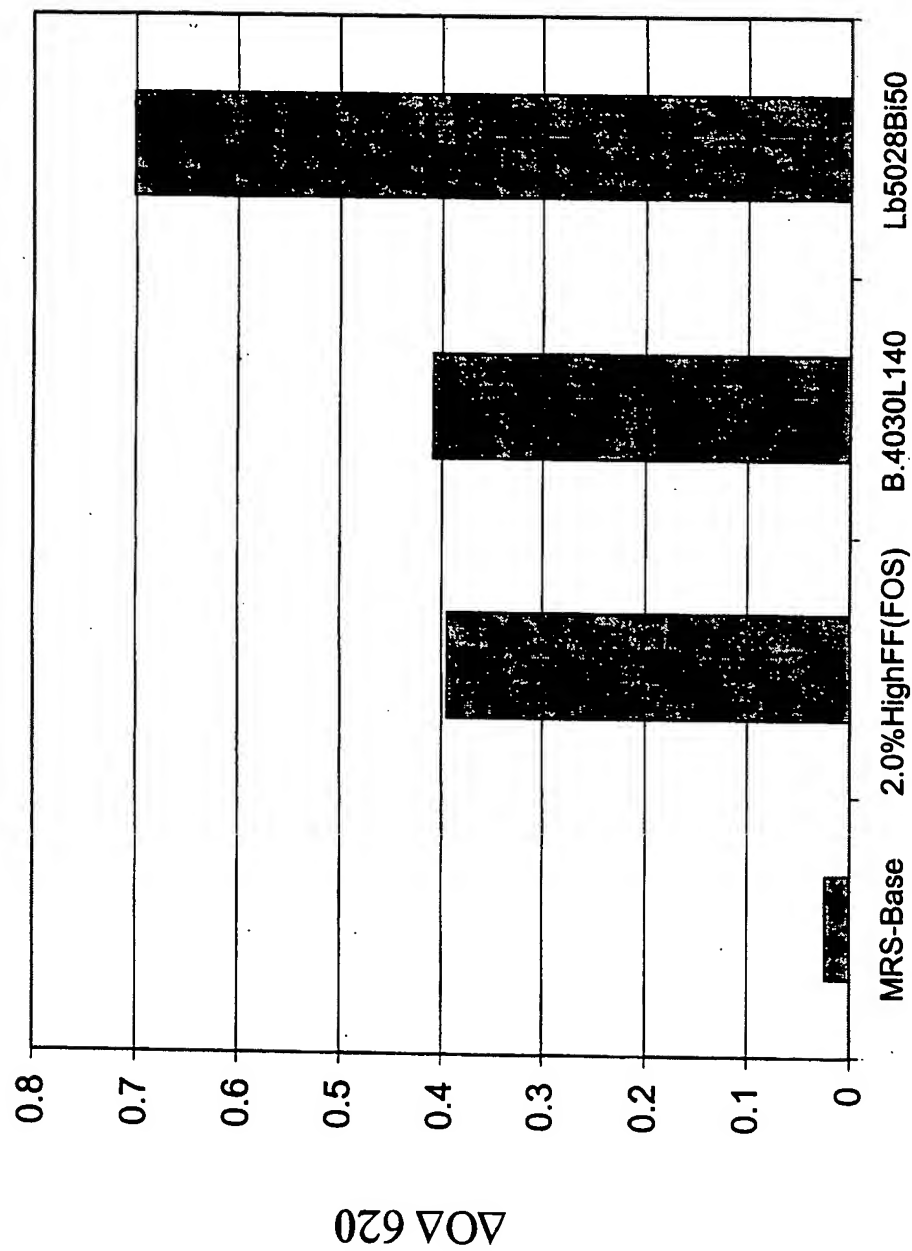
# **Growth of *Lactobacillus acidophilus* ATCC 4357 on different substrates 11/30/00.**



Time(24h)

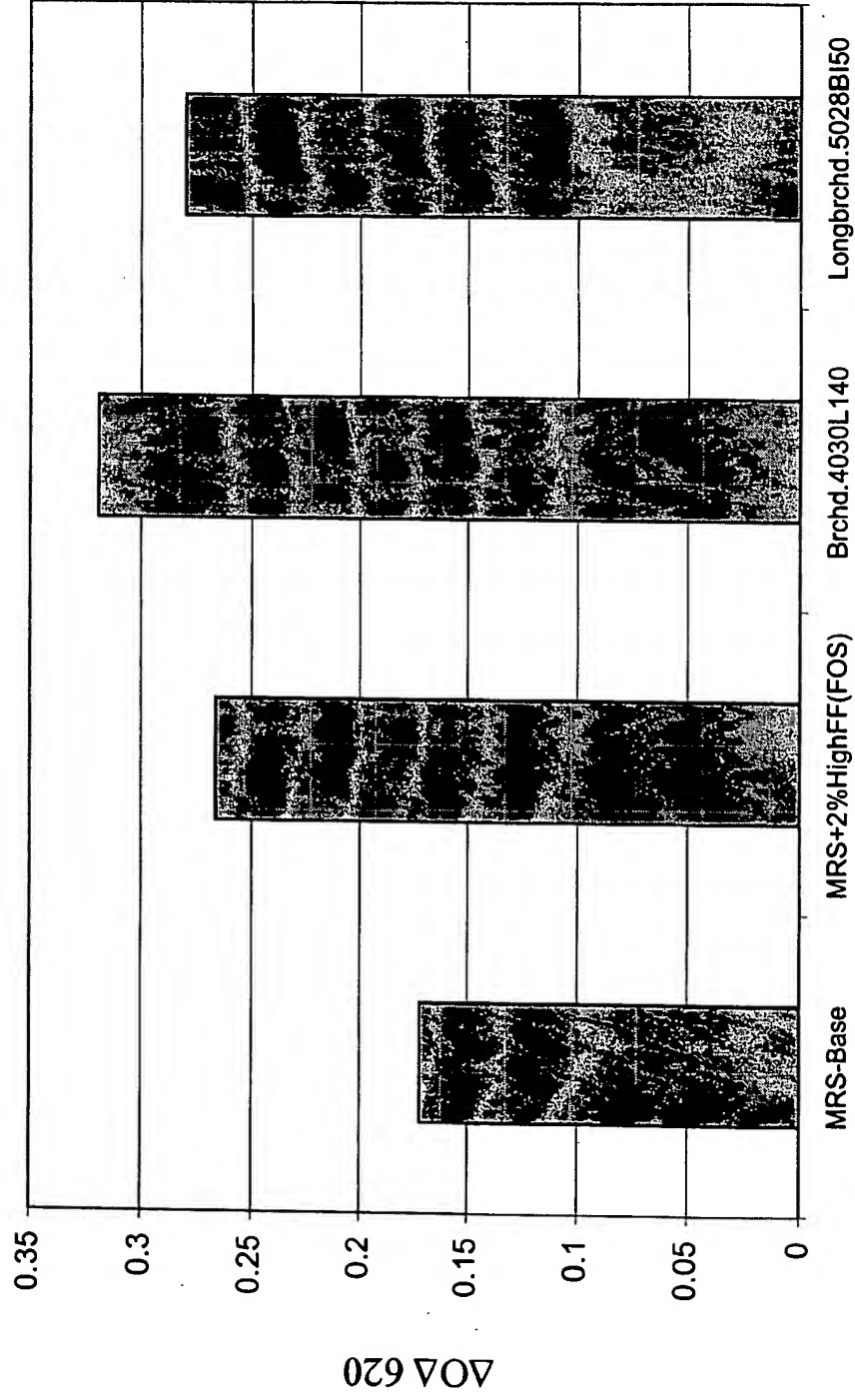
FIG. 1

2/44  
Growth of *Lactobacillus johnsonii* ATCC 33200 on  
different substrates



Time(8)  
FIG. 2

3/44  
 Growth of *Lactobacillus amylovorus* ATCC 33620 on  
 different substrates 12/8/00.

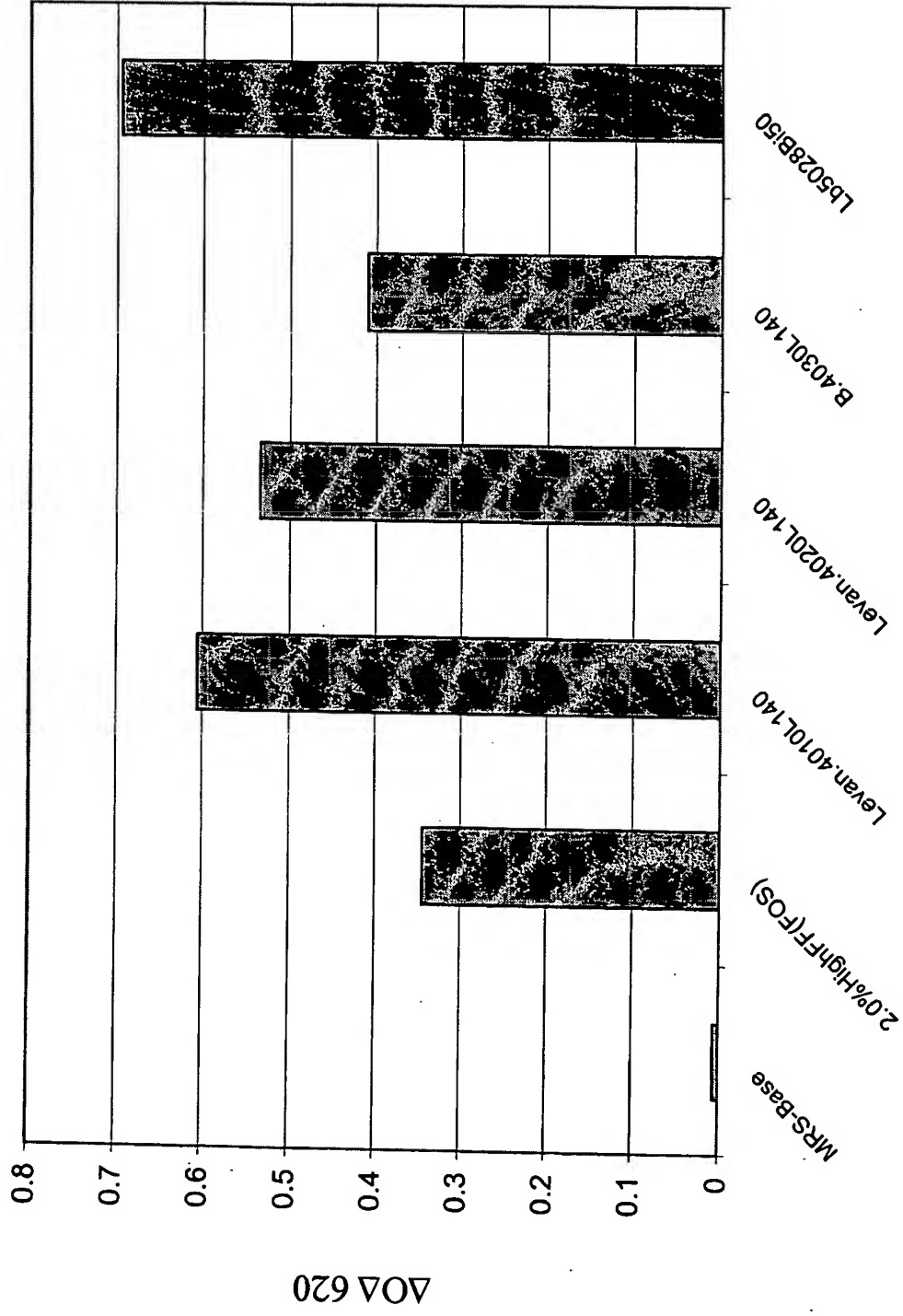


Time(6h)

FIG. 3

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# Growth of *Lactobacillus amylovorus* ATCC 33198 on different substrates.

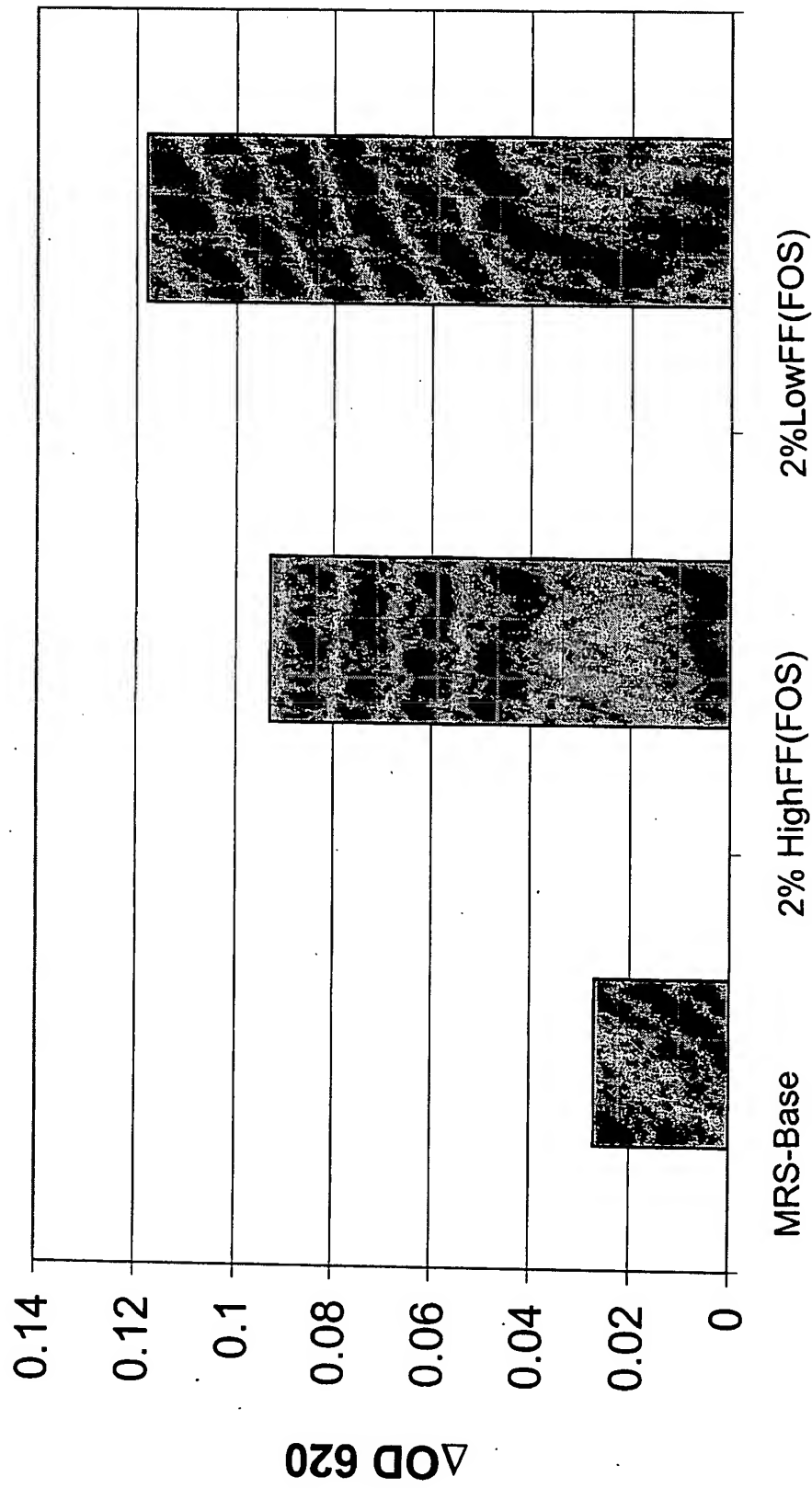


Time(24h)

FIG. 4

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# Growth of *Lactobacillus johnsonii* ATCC 33200 on different substrates



Time(8h) FIG. 5

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# Growth of *Lactobacillus plantarum* ATCC 4008 on different substrates 5/23/01.

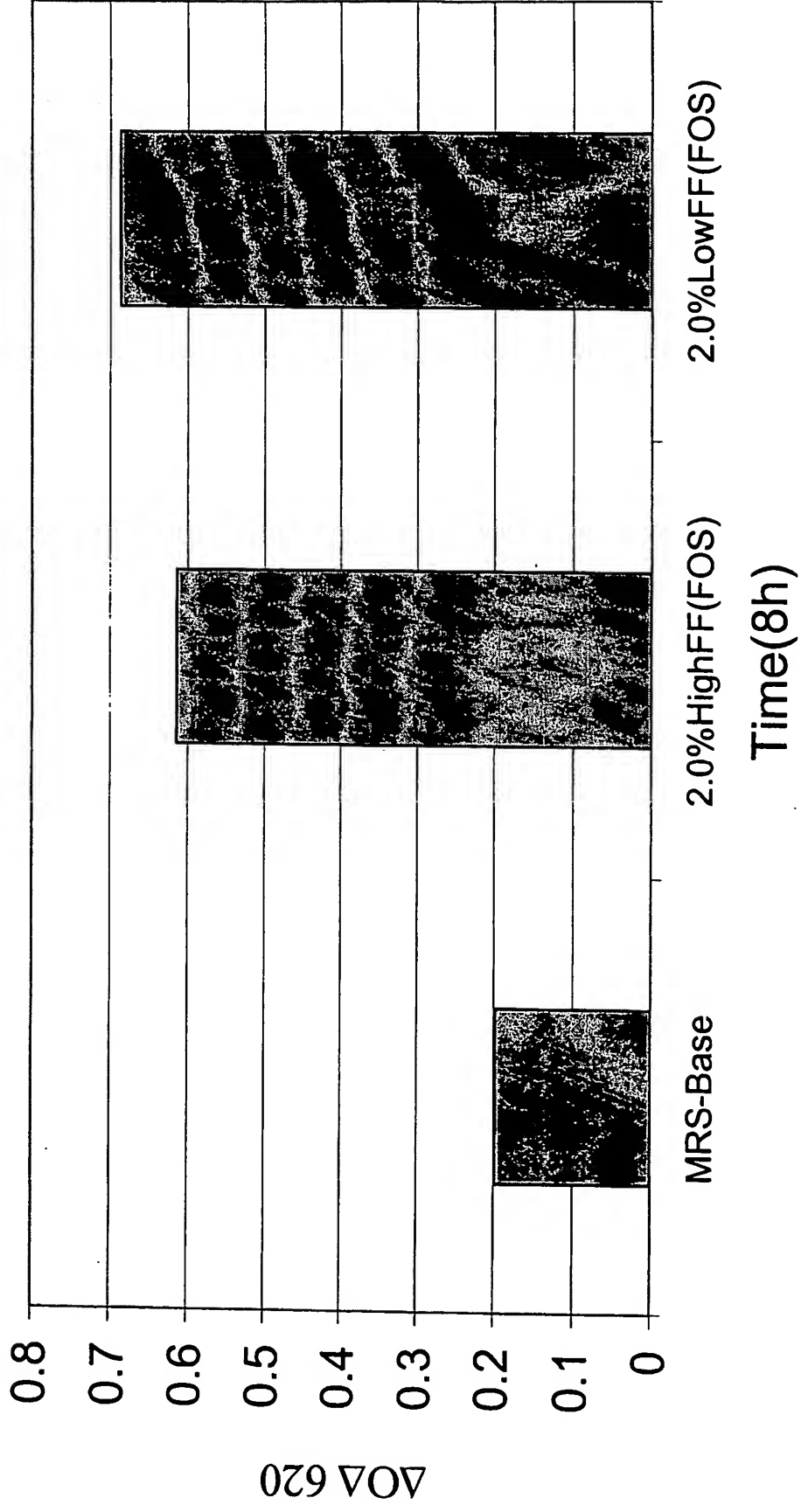
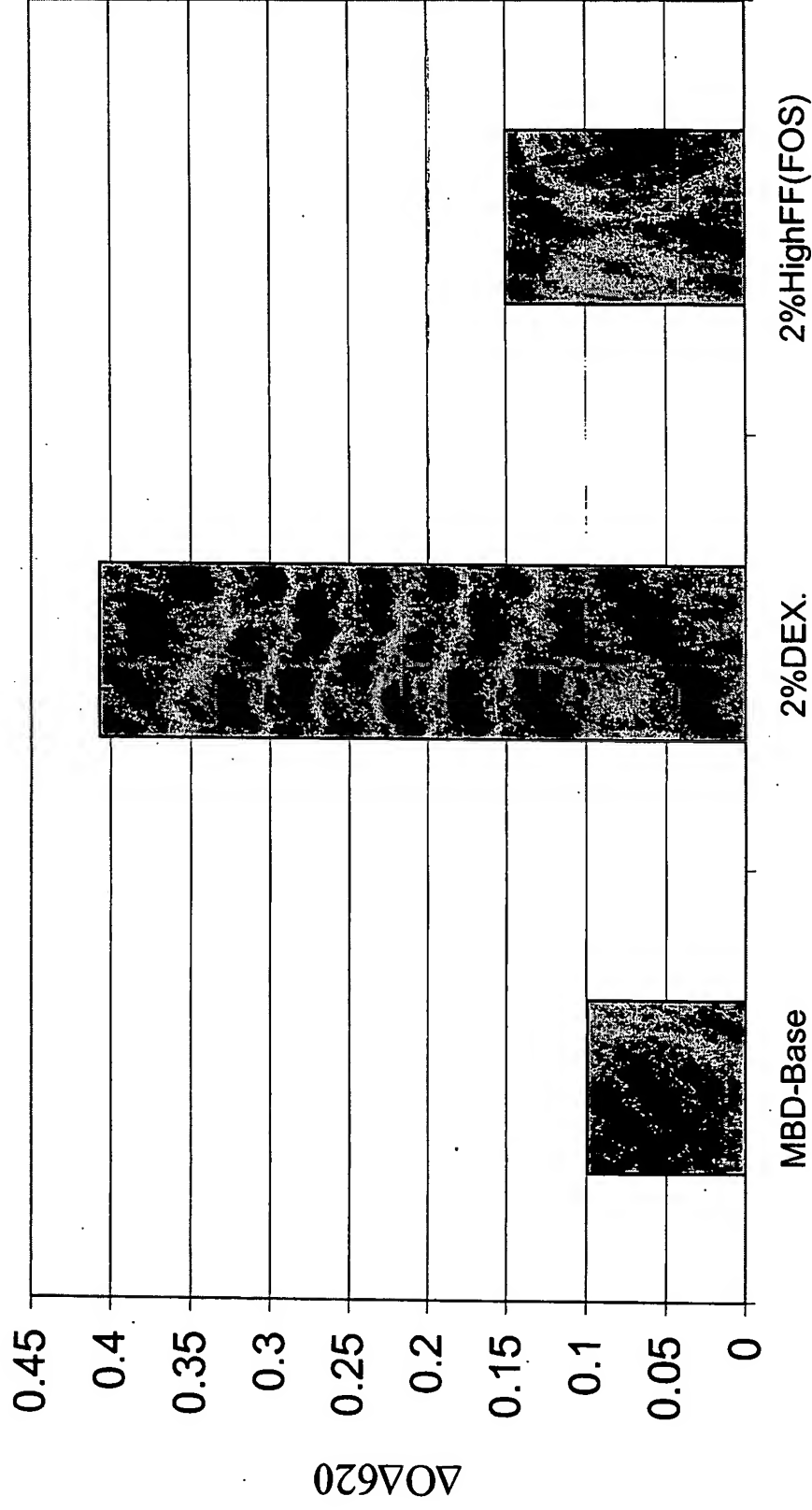


FIG. 6

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# Growth of Escherichia coli ATCC 23502(05-K4) on different substrates



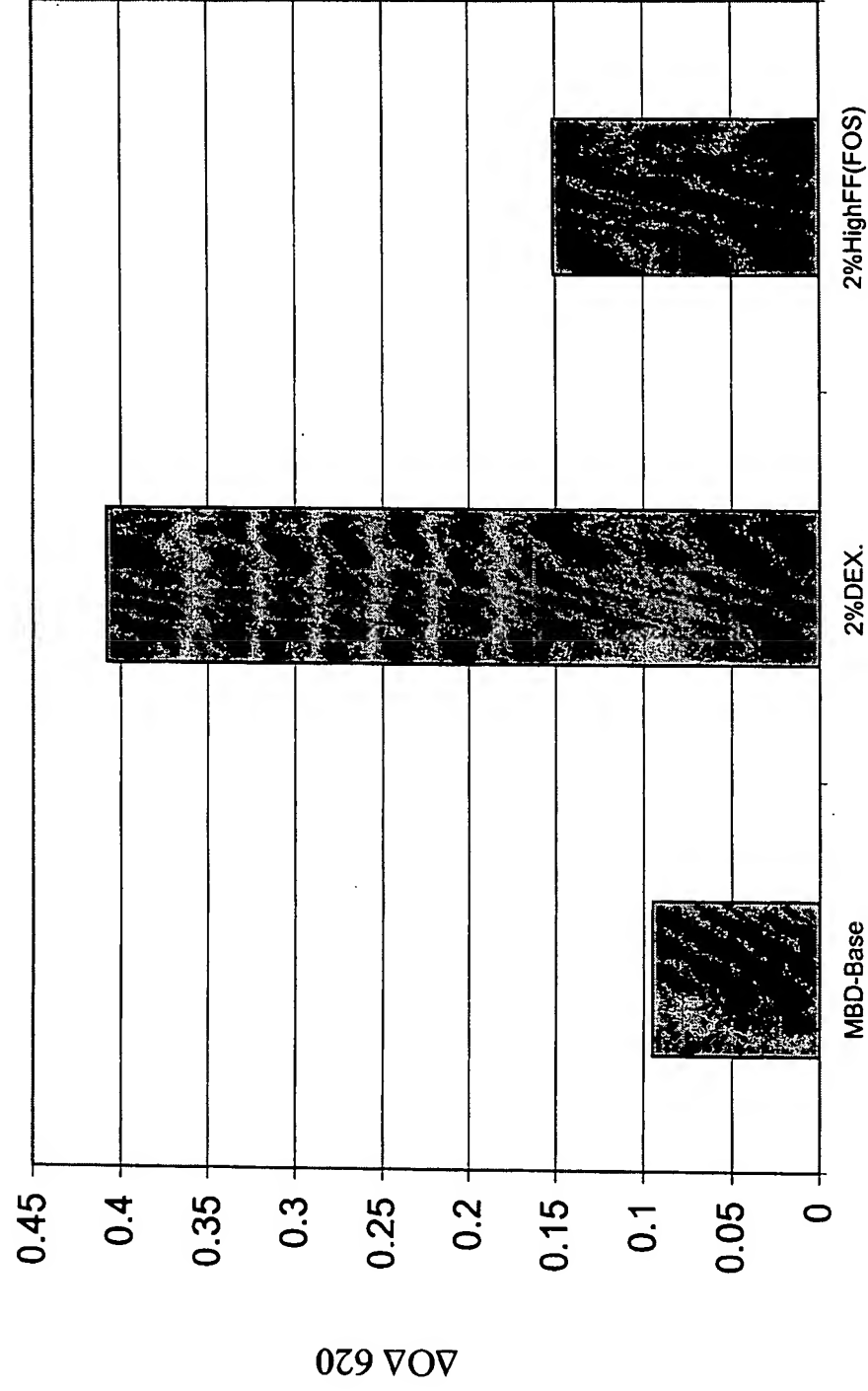
Time(24h)

FIG. 7



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# Growth of Escherichia coli U1-41(05-K4) on different substates 1/4/01.

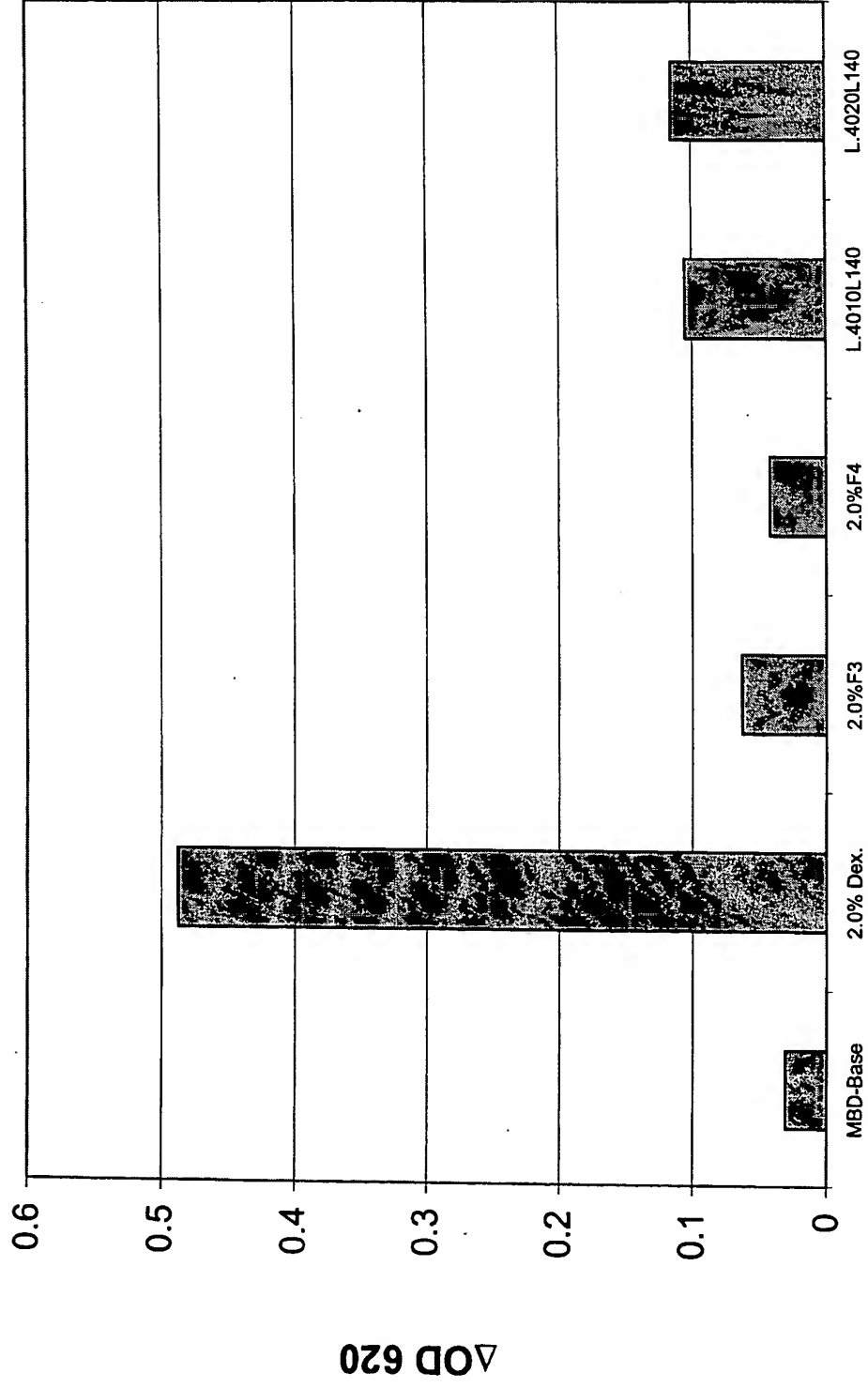


Time(24h)

FIG. 8

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# Growth of *Escherichia coli* O157:H7(021901-1) on different substrates

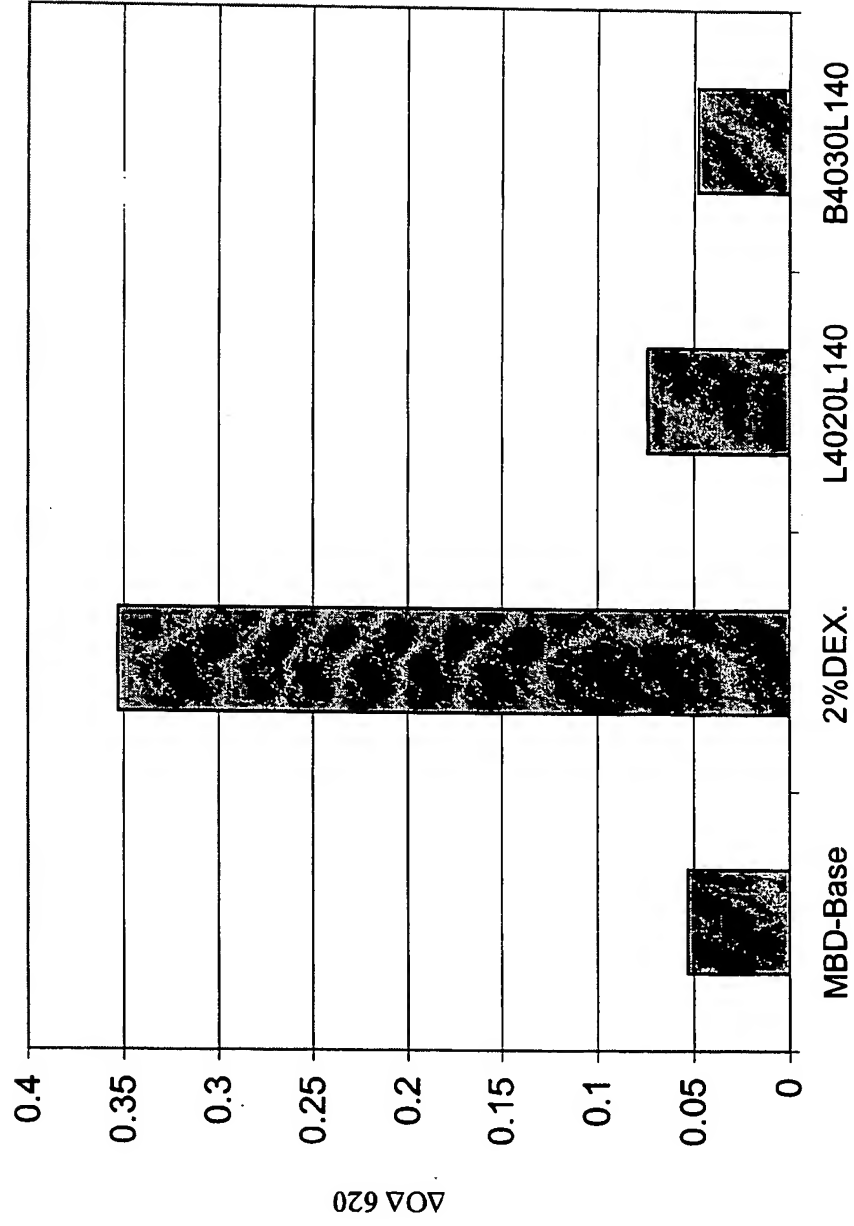


Time(8)

FIG. 9

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# Growth of Salmonella group B (021901-5) on different substrates 3/9/01.



Time(8h)

FIG. 10

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# Levan Distribution - Single Trapping Experiment Versus Previous Averages

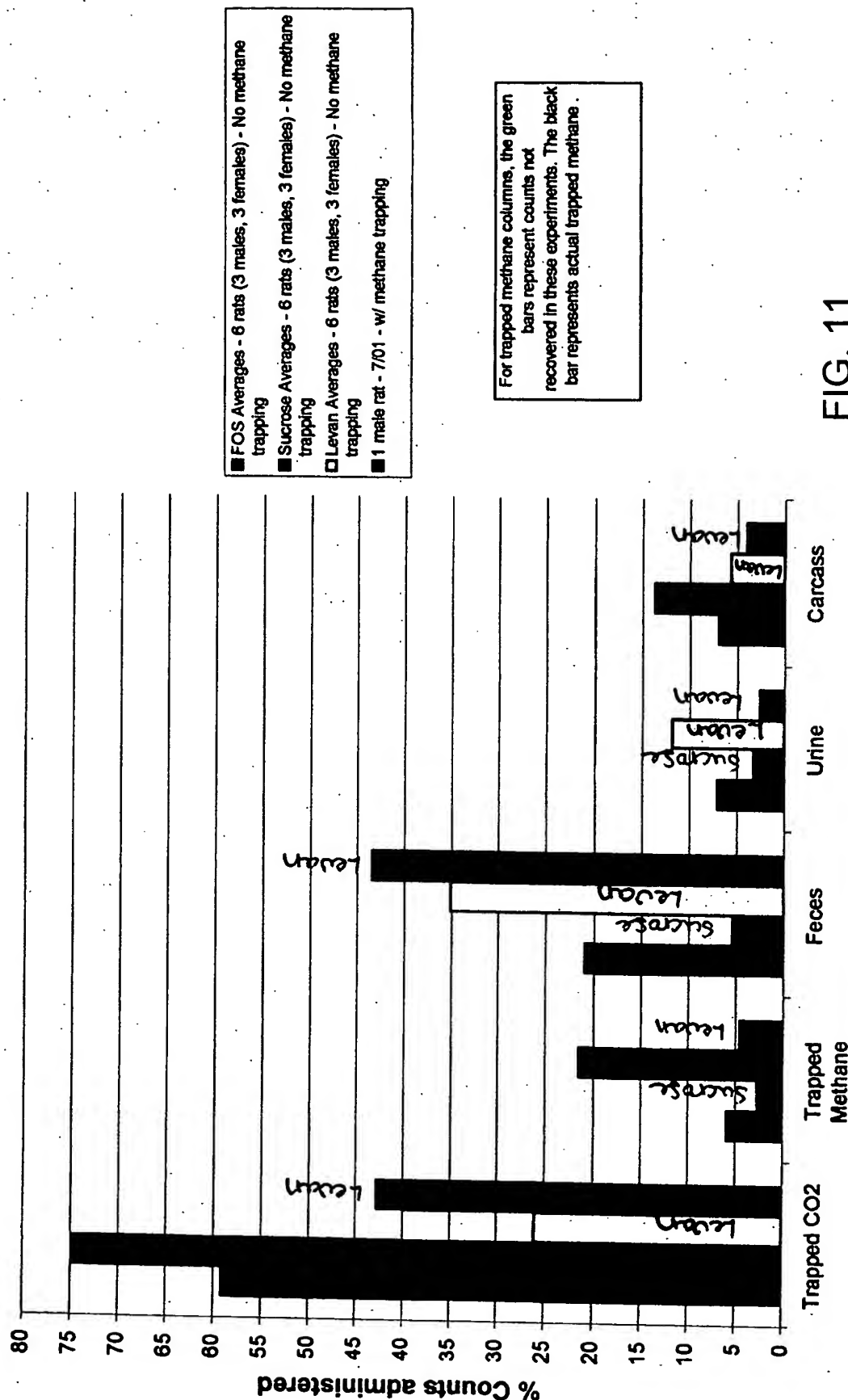


FIG. 11

12/44  
Levan 4010LI40 @50 oC

5

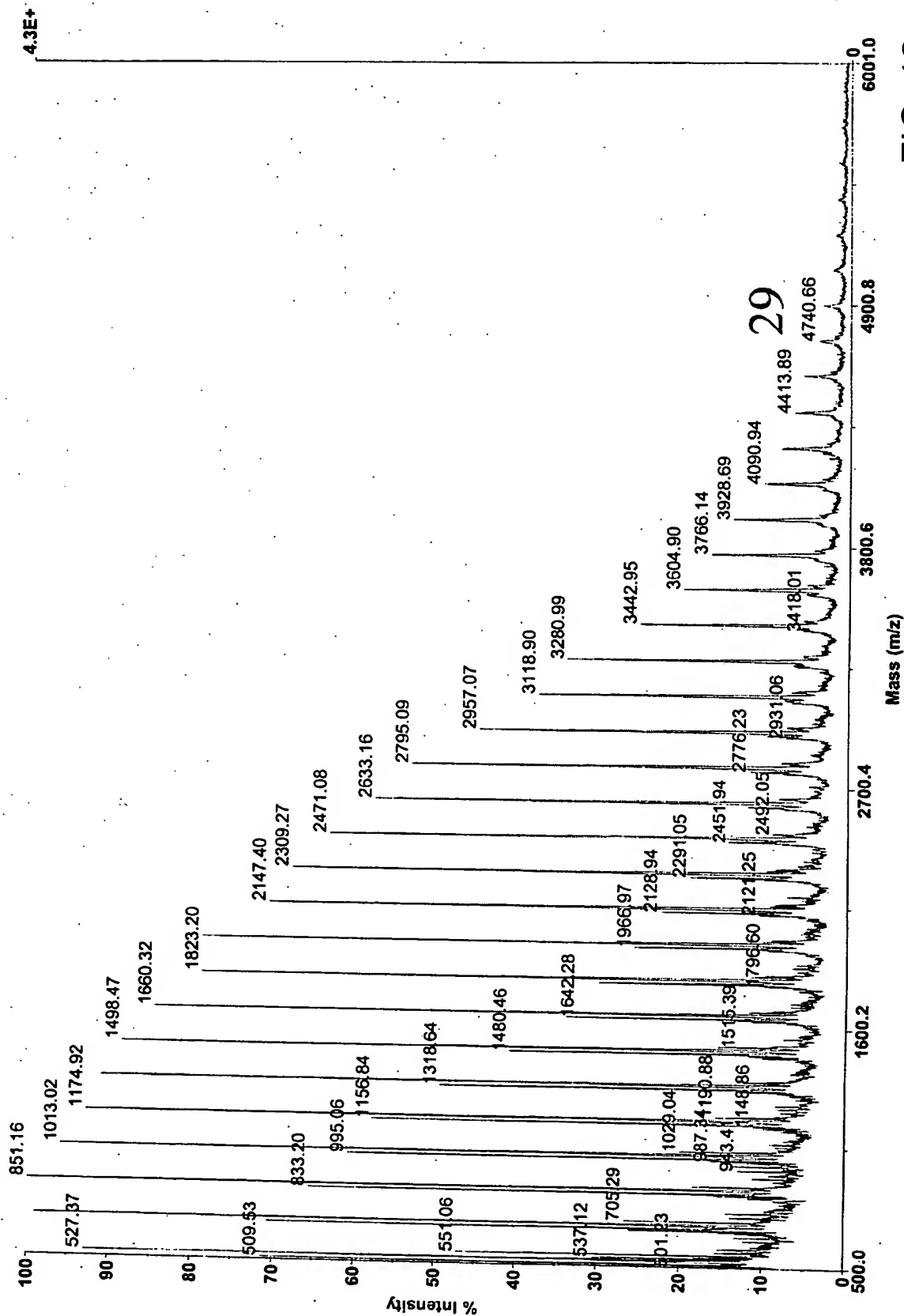


FIG. 12

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Short-branched fructan 4030LI40 @ 2:1 FOS:Sucrose

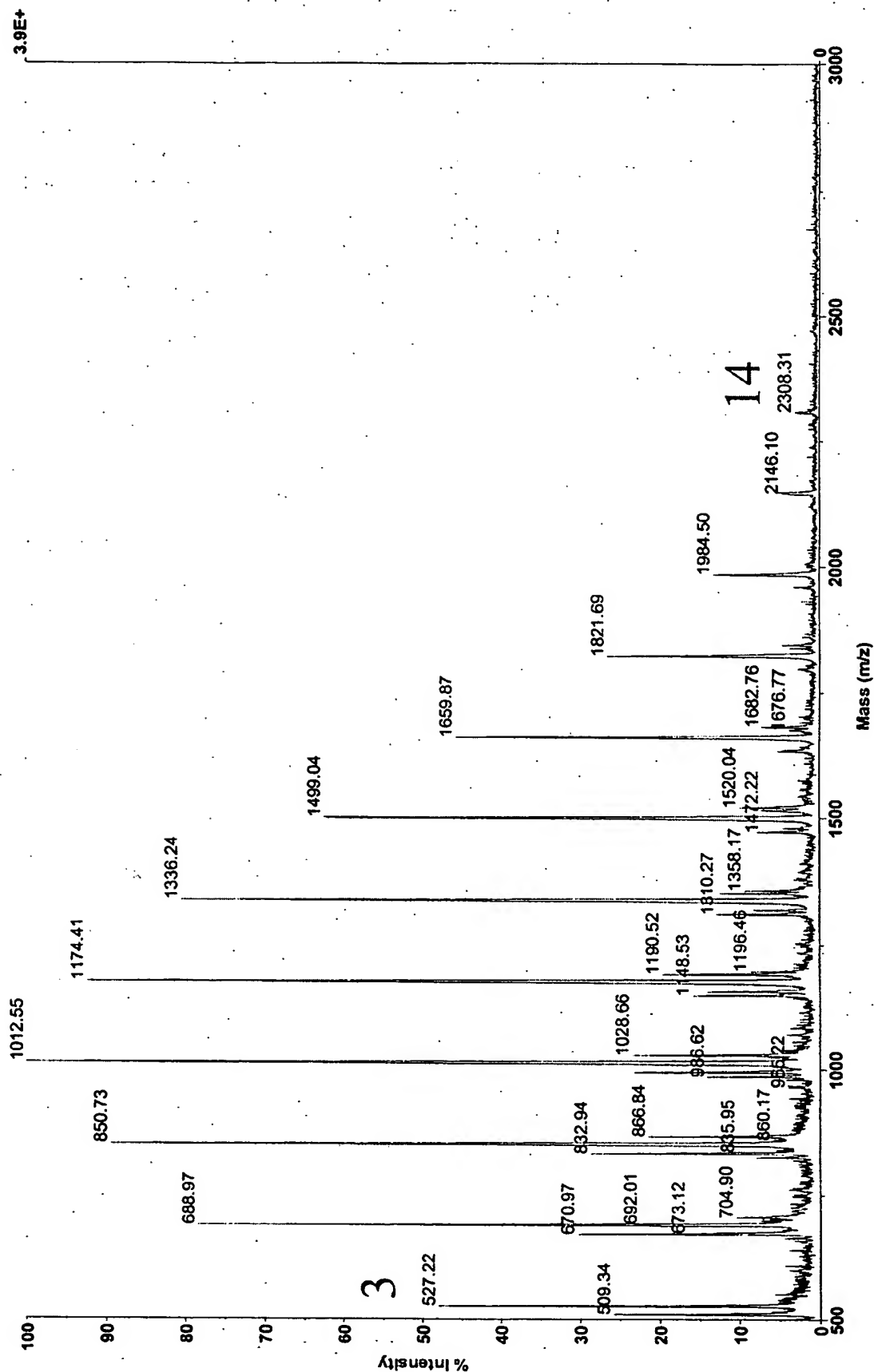


FIG. 13

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FOS

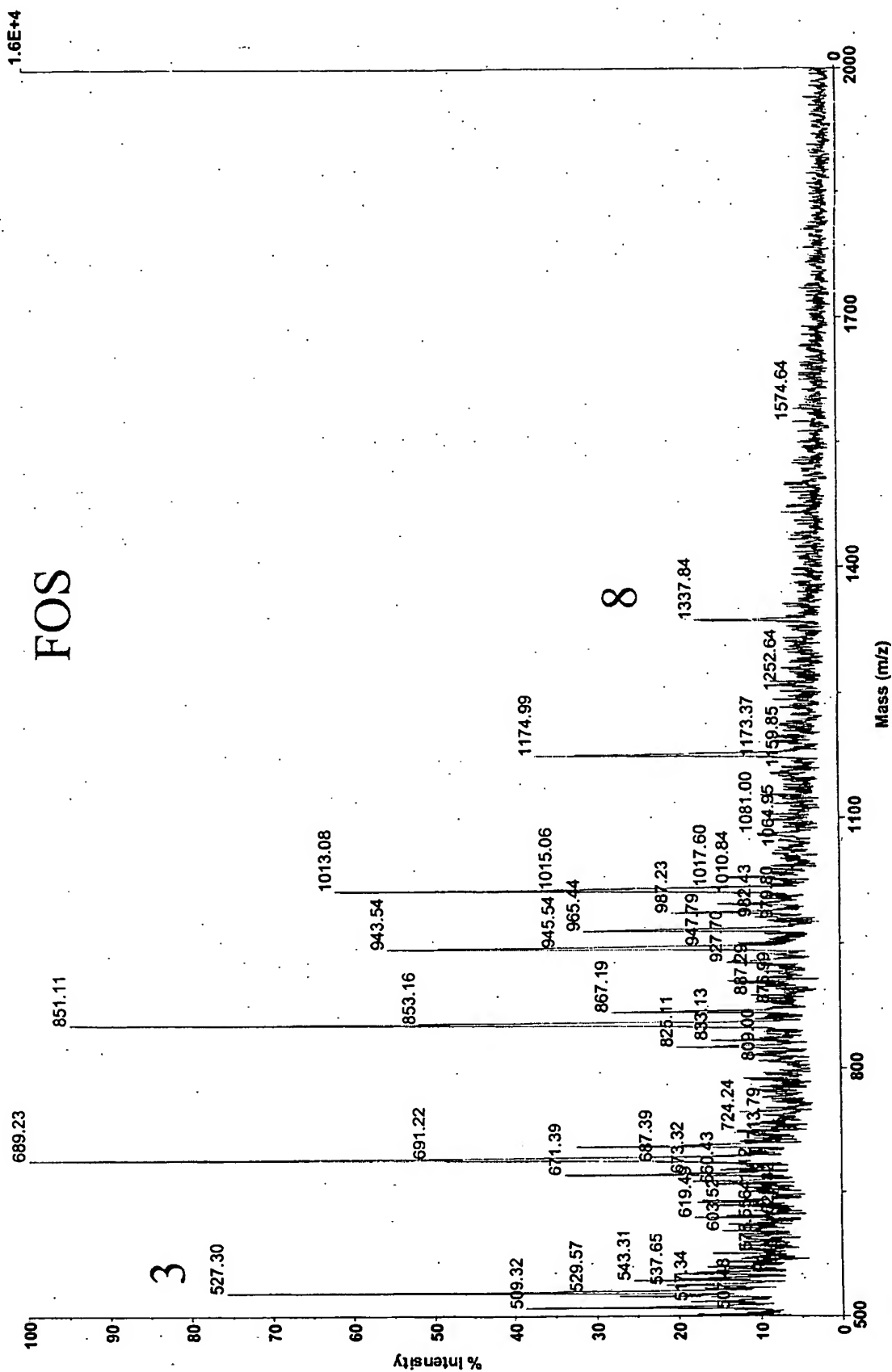


FIG. 14

Levan 4020LI40 @ 30 °C

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FIG. 15A

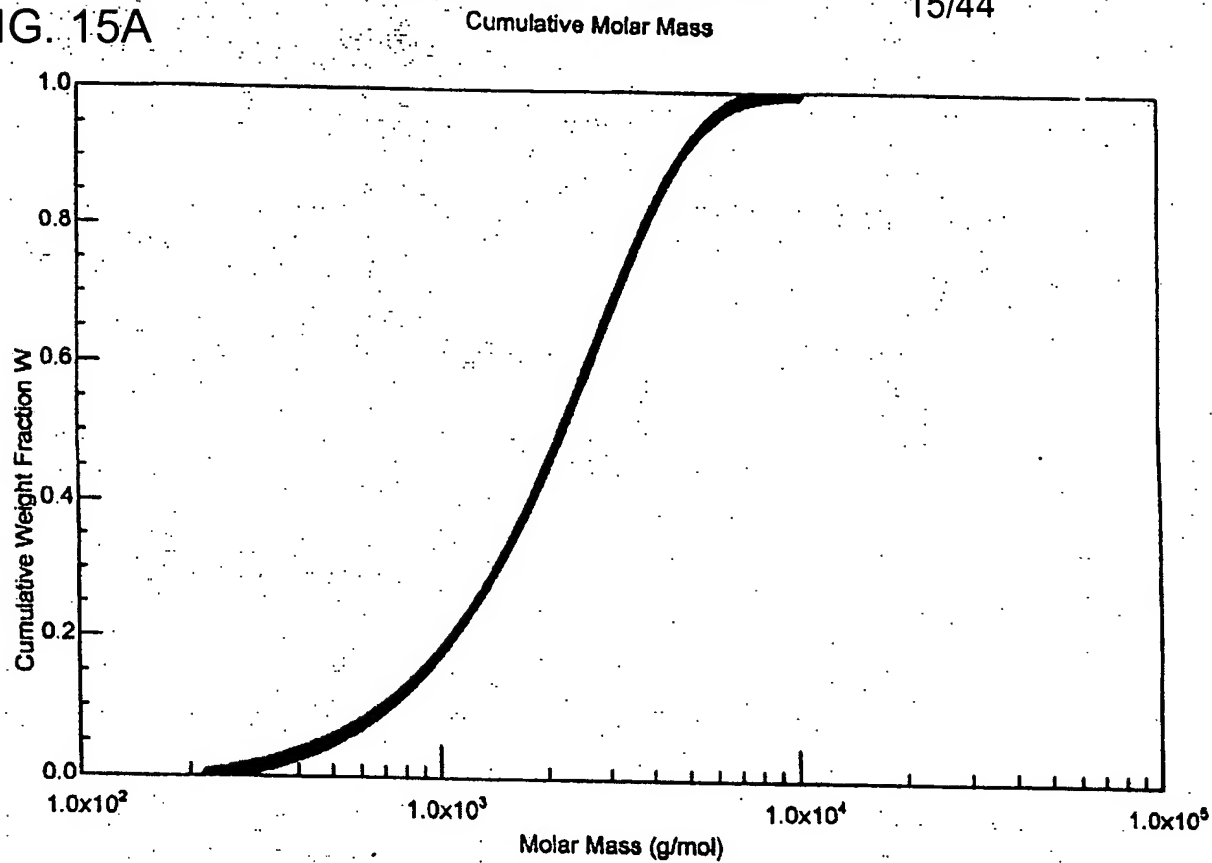


FIG. 15B

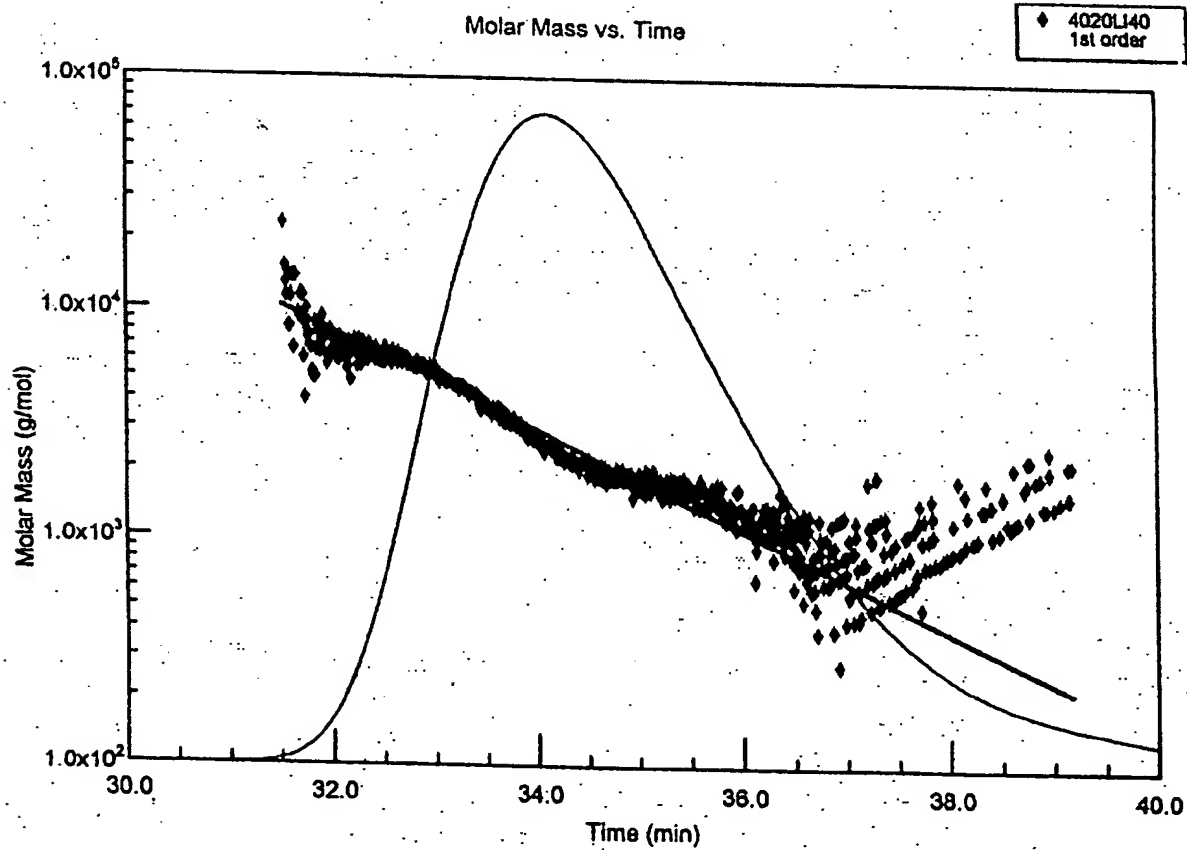




FIG. 16A

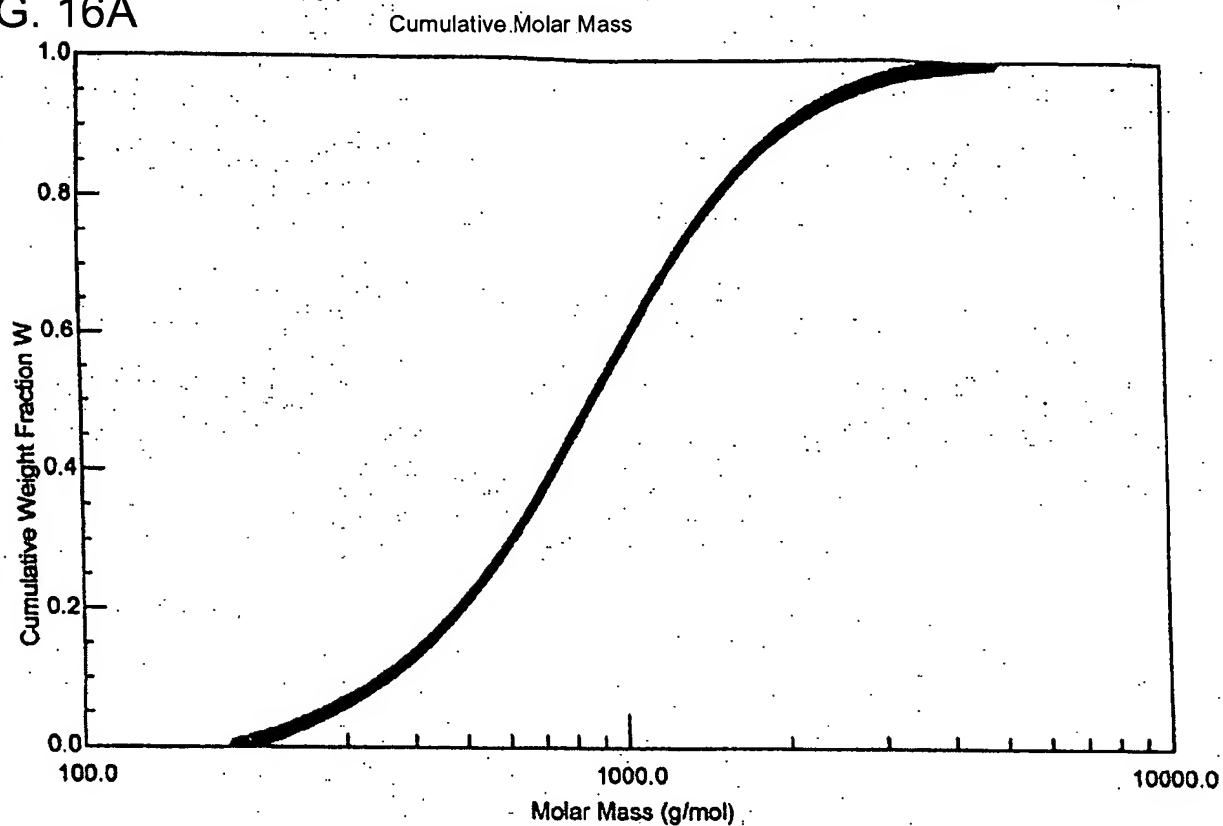
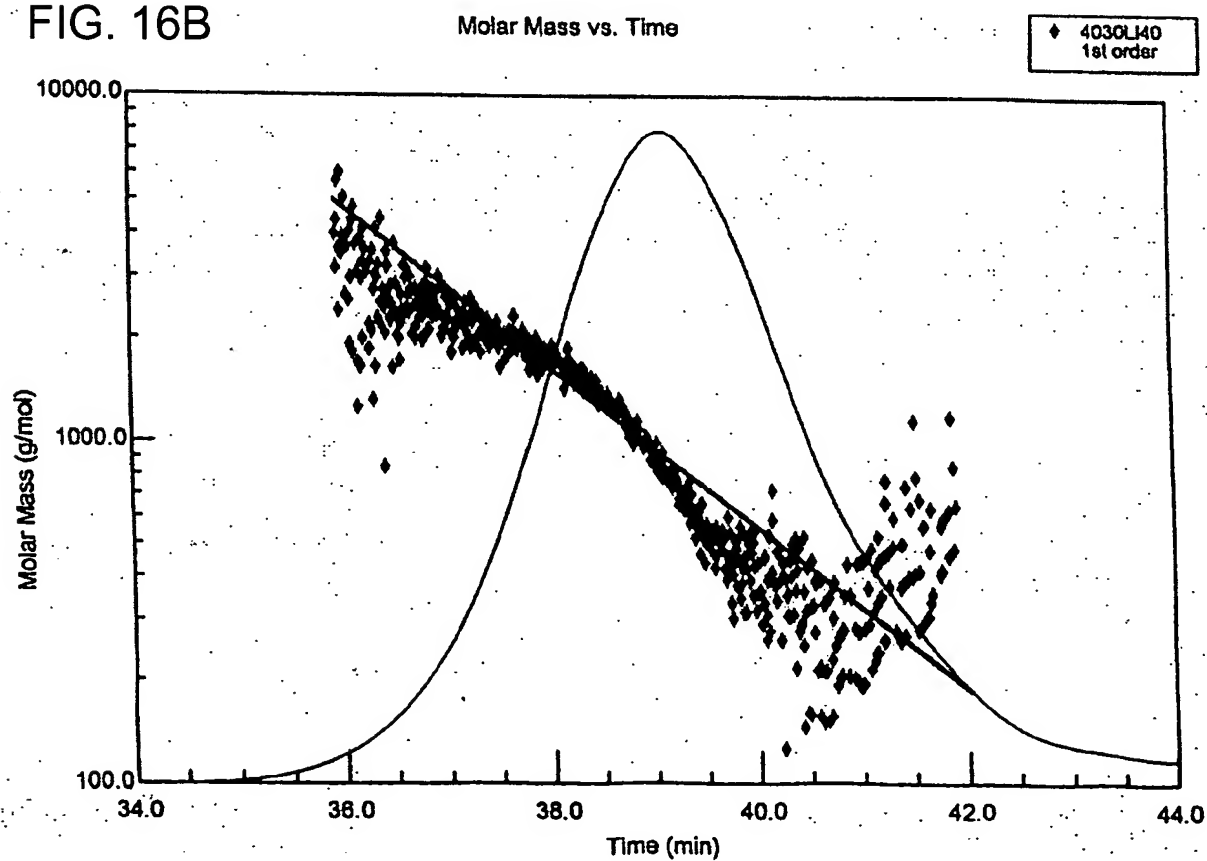
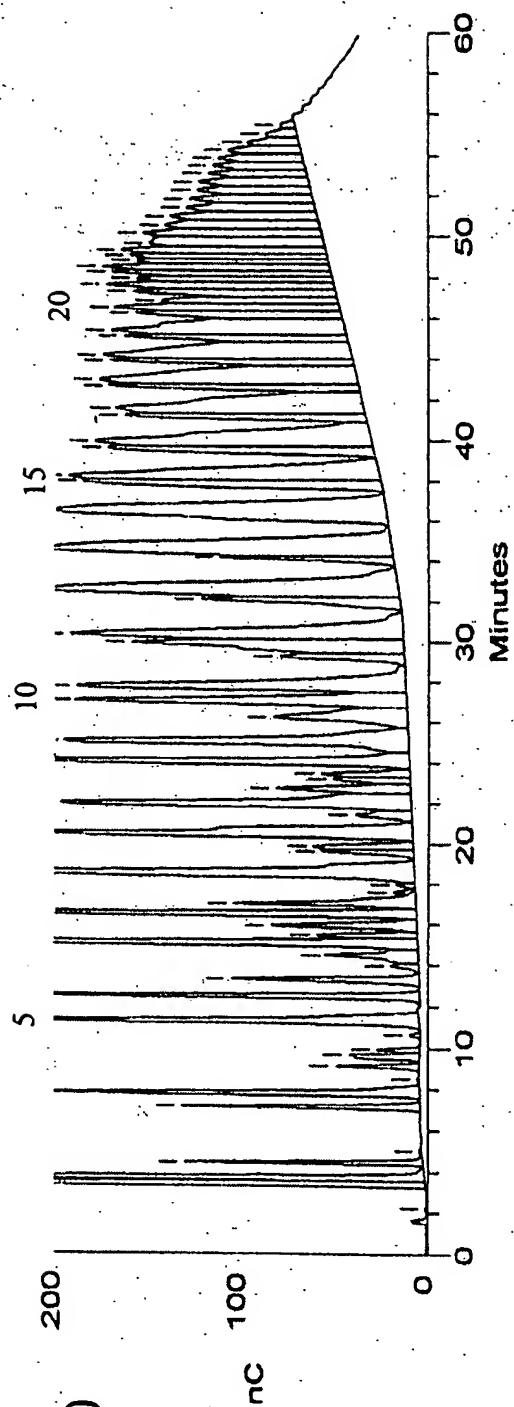


FIG. 16B



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Levan 4010LI40  
@ 50°C



Levan 4020LI40  
@ 30°C

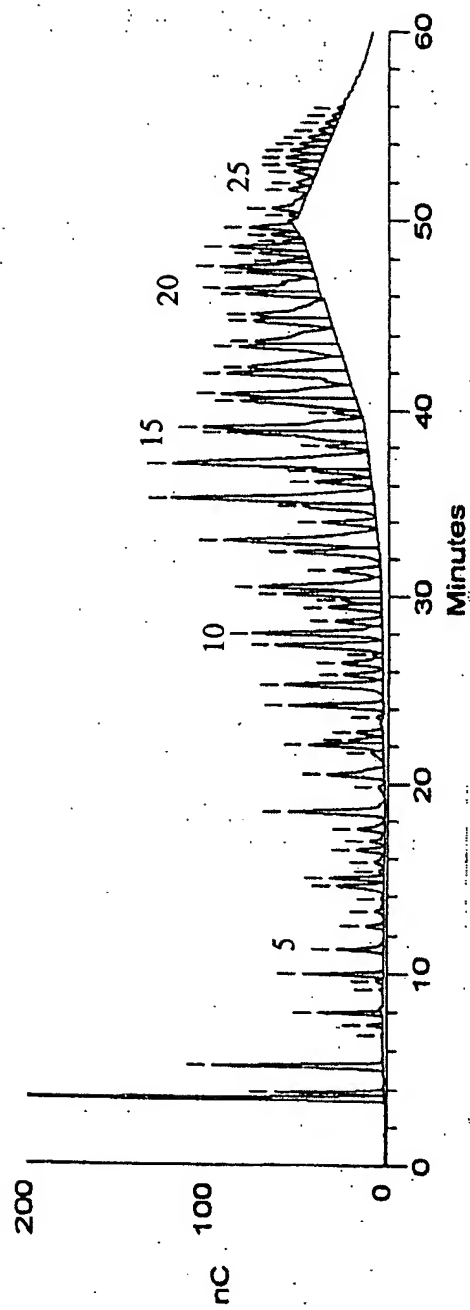
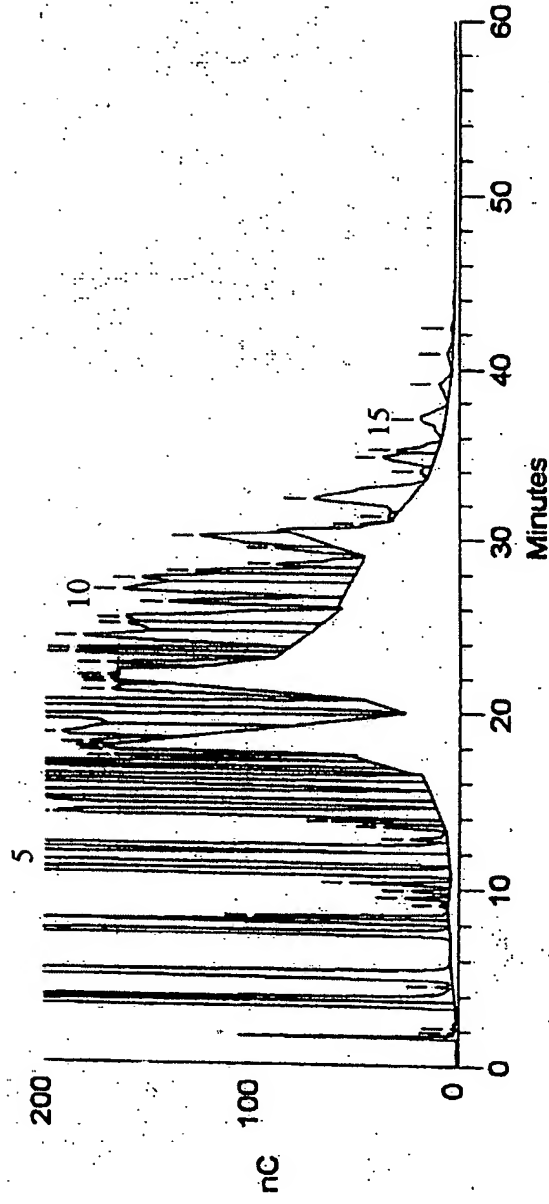


FIG. 17

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Short-branched fructan  
4030LI40

2:1 FOS:sucrose



Long-branched fructan  
5028BI50

1:1 FOS:sucrose

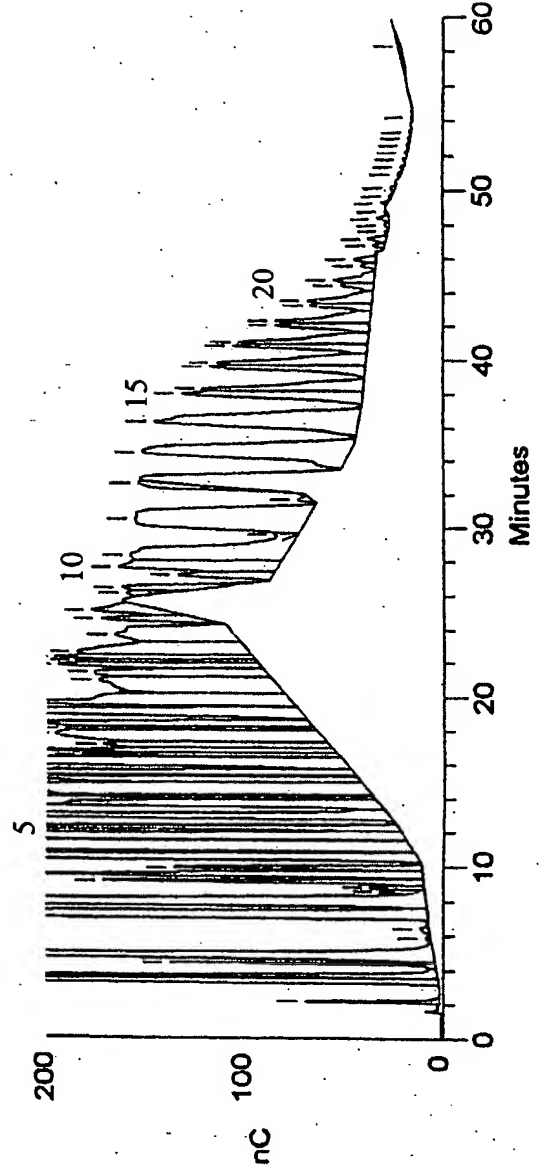


FIG. 18

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FOS and branched levan metabolism by *Lactobacillus acidophilus* ATCC 4357 using MRS media w & w/o 2% sugars at 37 °C 12% CO<sub>2</sub> incubation 11/30/00.

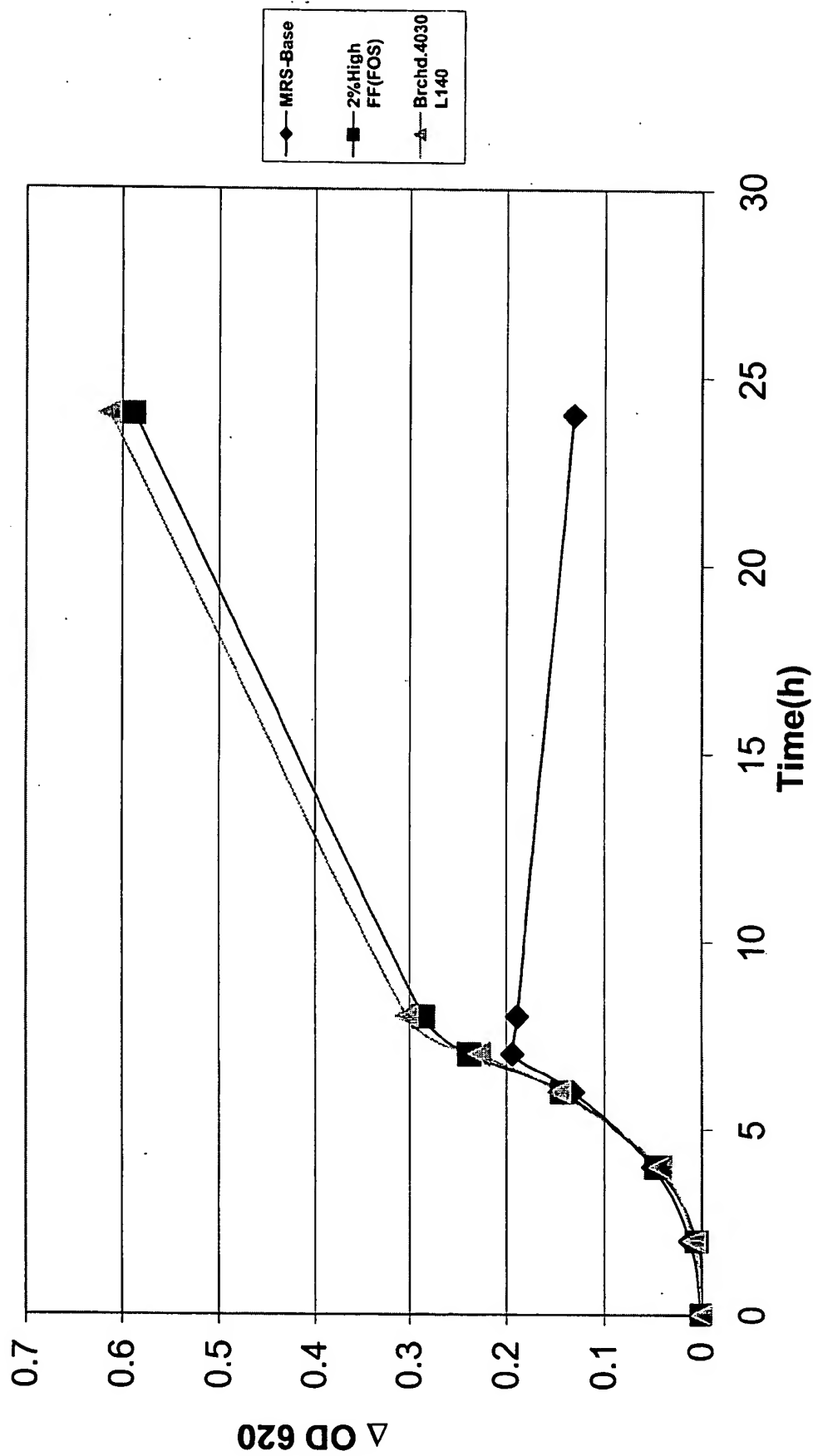


FIG. 19

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FOS and branched levan metabolism by *Lactobacillus amylovorus* ATCC 33620  
using MRS media w & w/o sugars at 37 °C 5% CO2 incubation 12/8/00.

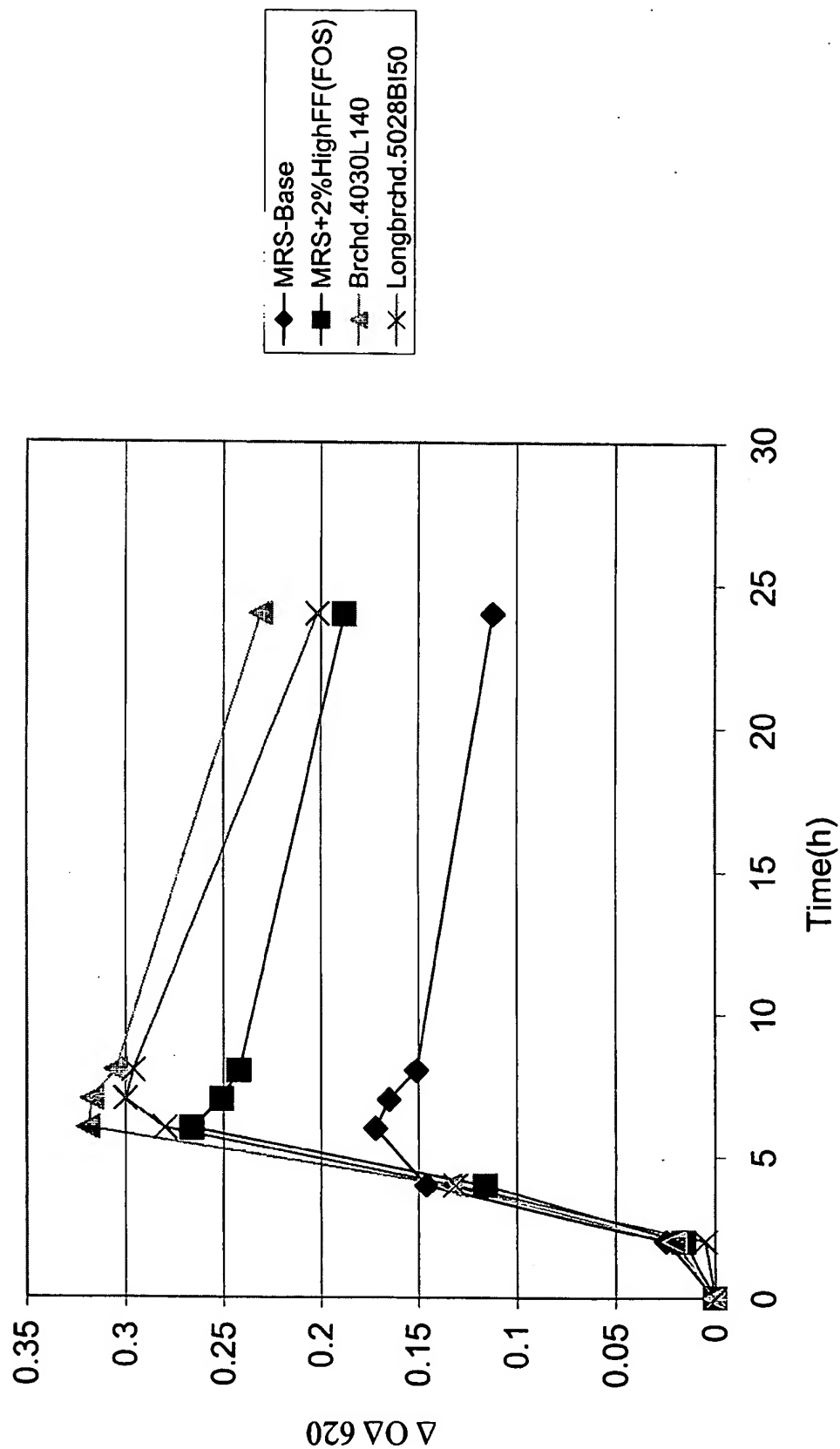


FIG. 20

FOS and branched levan metabolism by *Lactobacillus amylovorus* ATCC 33198 using MRS media w & w/o 2% sugars at 37°C 5% CO<sub>2</sub> incubation.

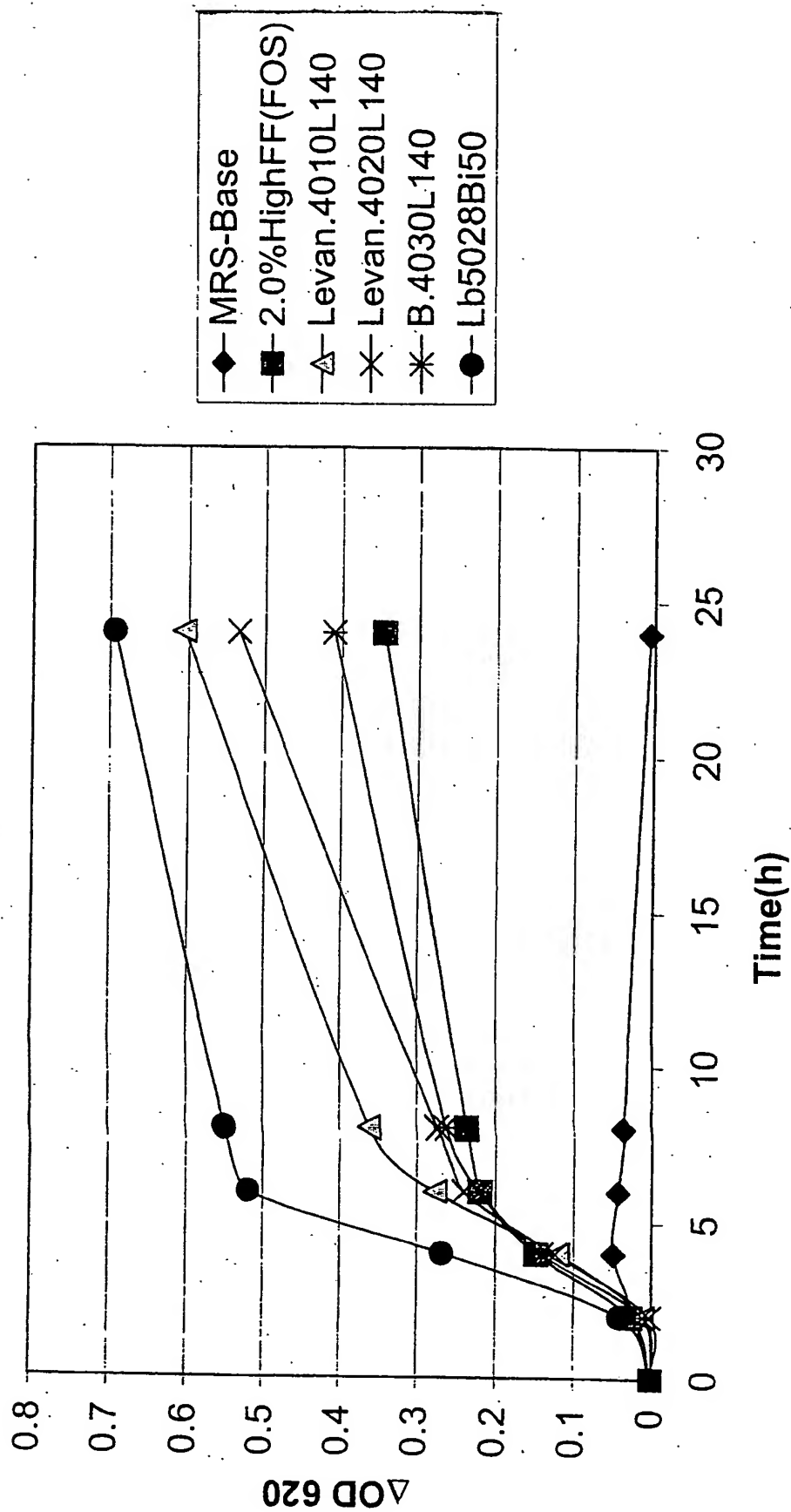


FIG. 21

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Fos metabolism by *Lactobacillus johnsonii* ATCC 33200 using MRS media w & w/o sugars at 37 °C 5% CO<sub>2</sub> incubation 5/23/01.

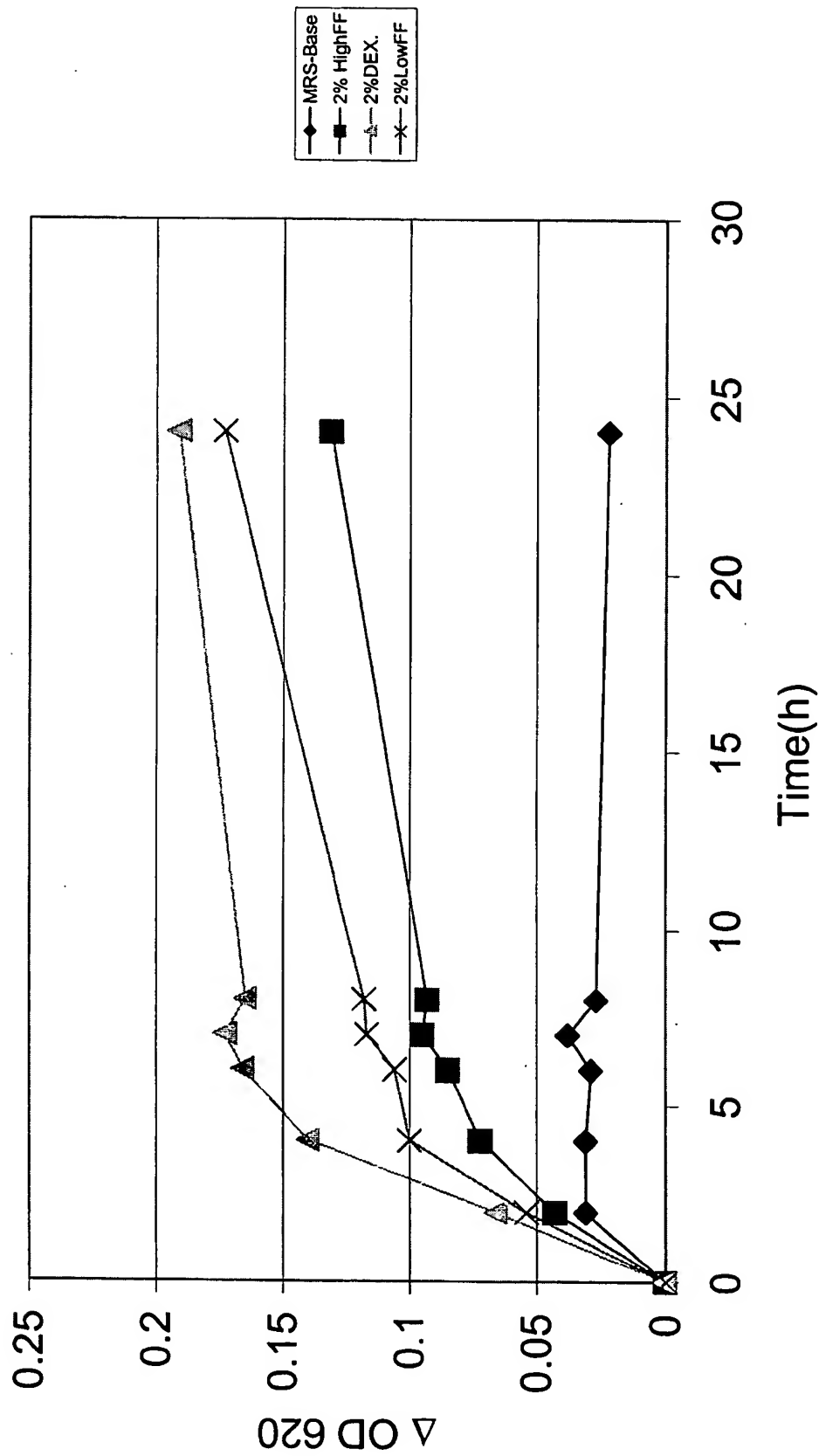


FIG. 22

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FOS metabolism by *Lactobacillus plantarum* ATCC 4008 using MRS media w & w/o sugars at 37 °C 5% CO<sub>2</sub> incubation 5/23/01.

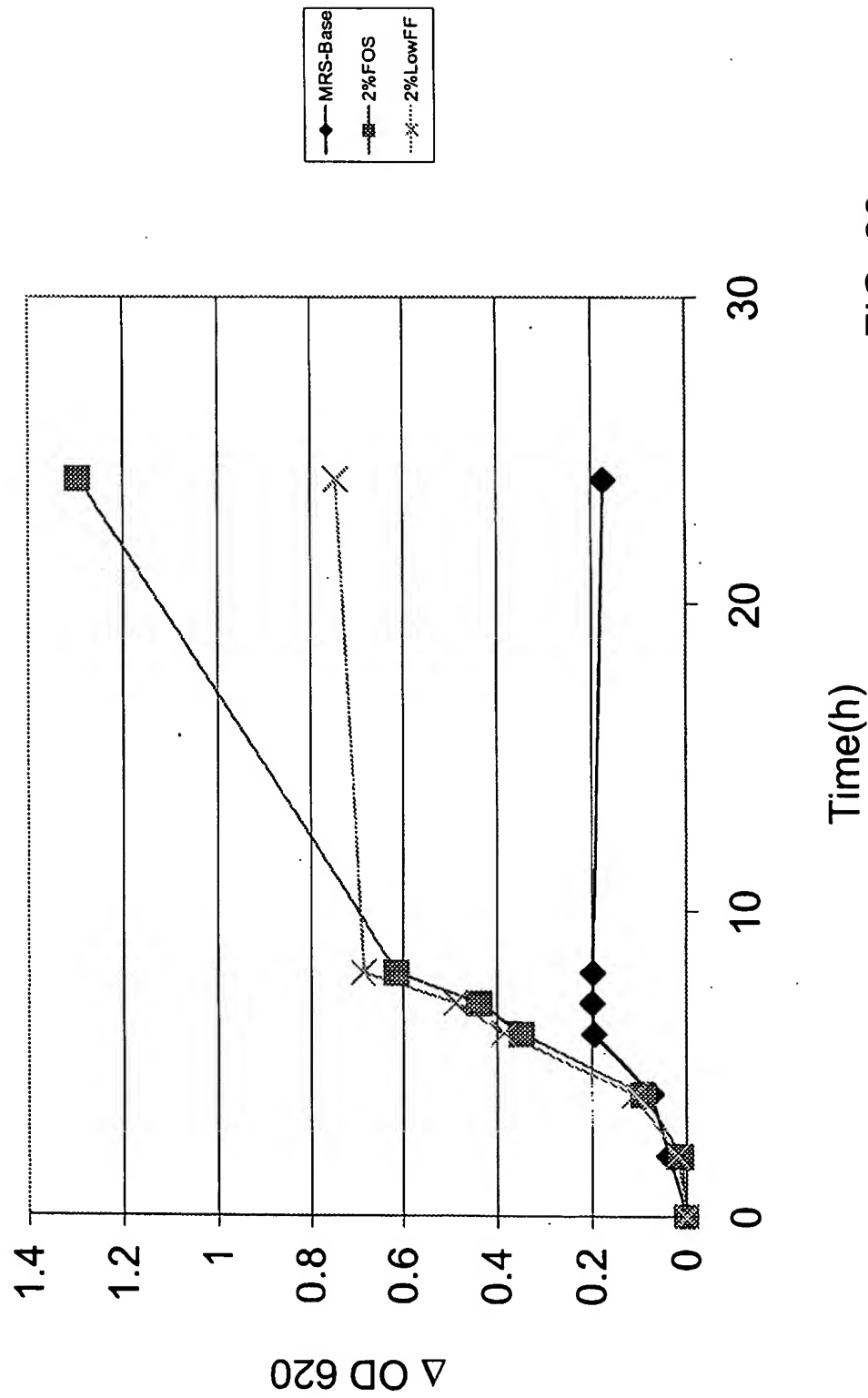


FIG. 23



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Sugar metabolism of *Escherichia coli* ATCC 23502 (O5-K4) using  
Minimal Broth Davis e & w/o sugars 1/4/01.

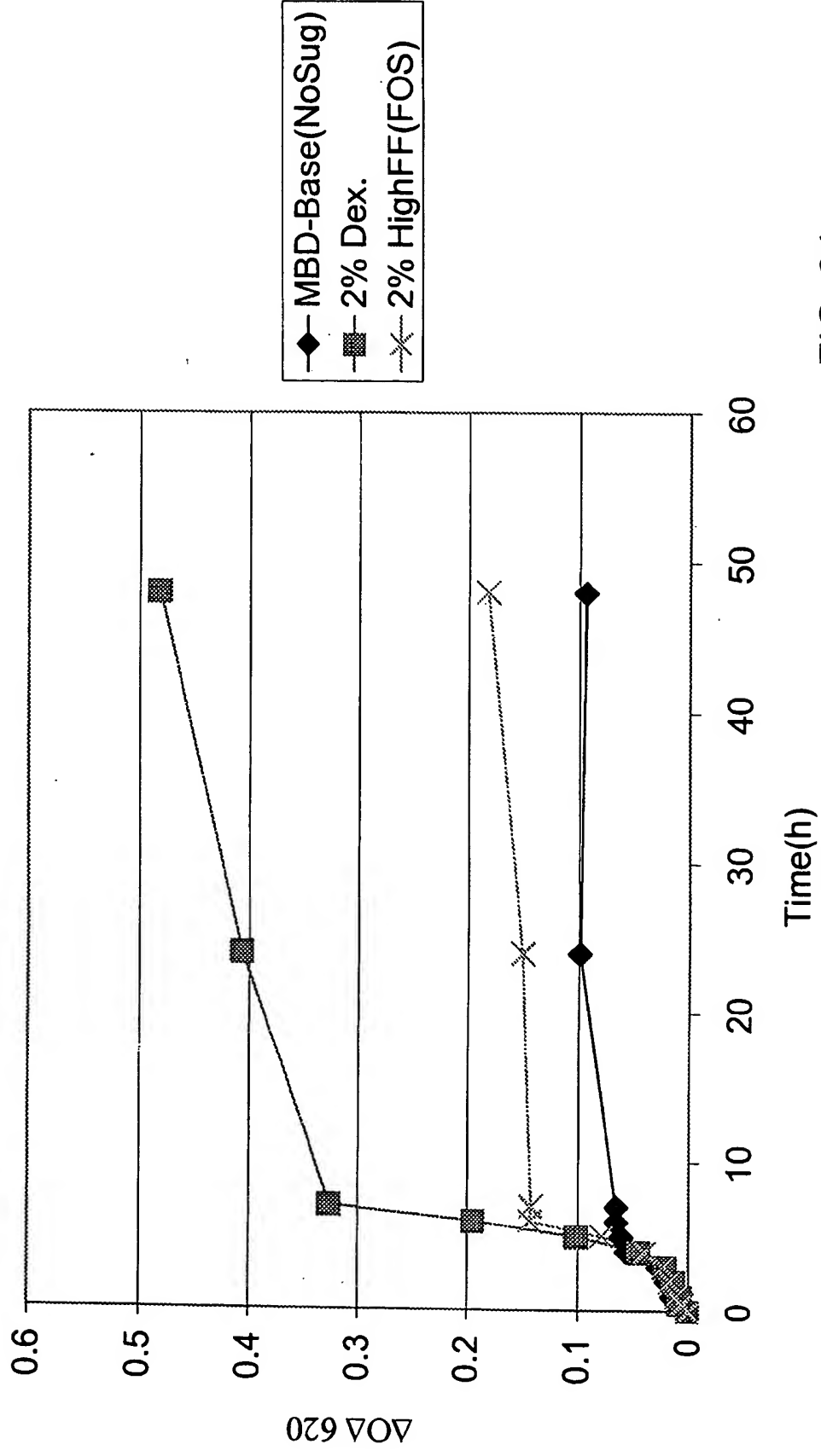


FIG. 24

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Sugar metabolism of *Escherichia coli* U1-41(05-K4) using Minimal  
Broth Davis w & w/o 2% sugars 1/4/01.

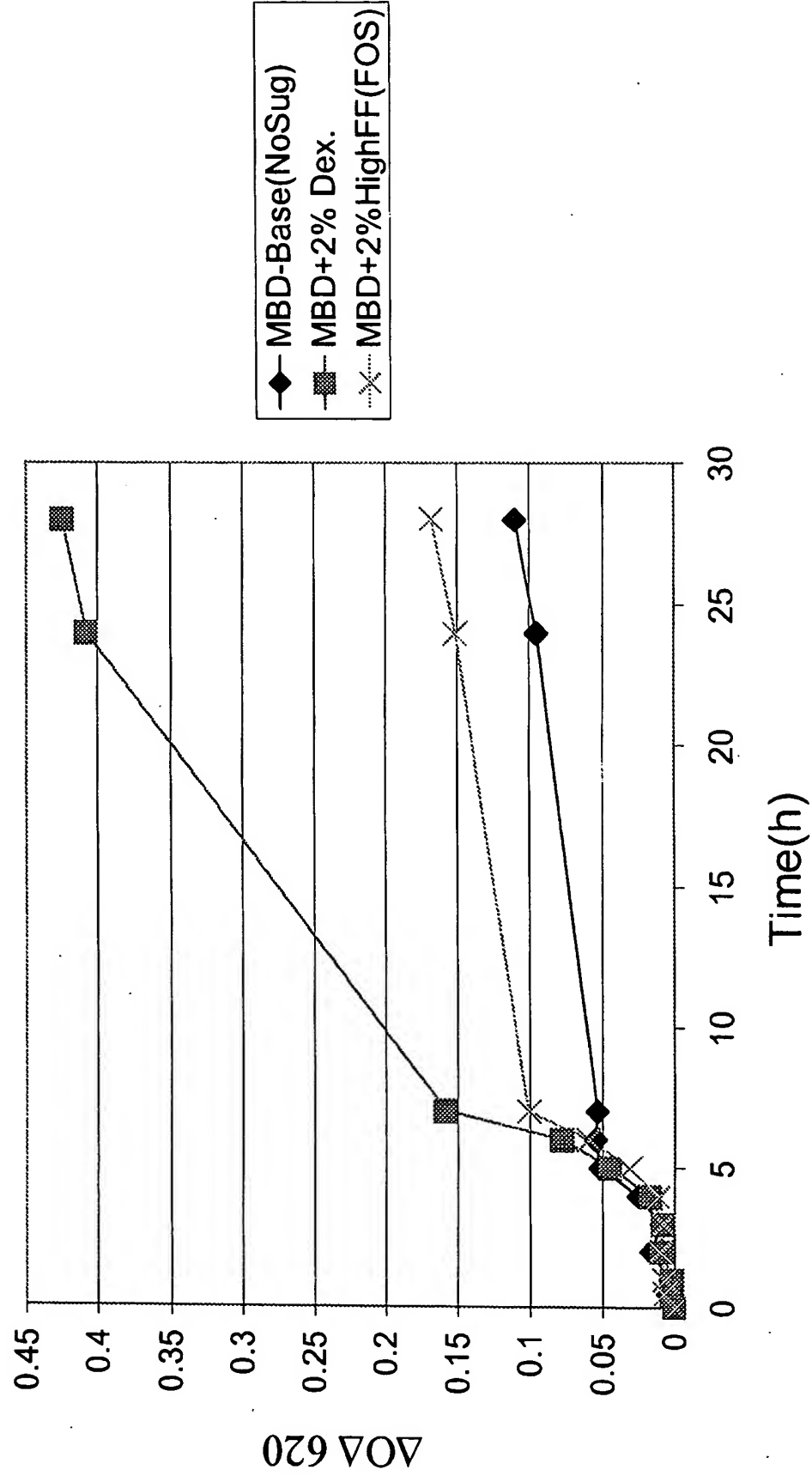


FIG. 25

FOS metabolism by *Escherichia coli* O157:H7(021901-1) using  
MBD media w & w/o sugars at 37 °C 5% CO<sub>2</sub> incubation 3/1/01.

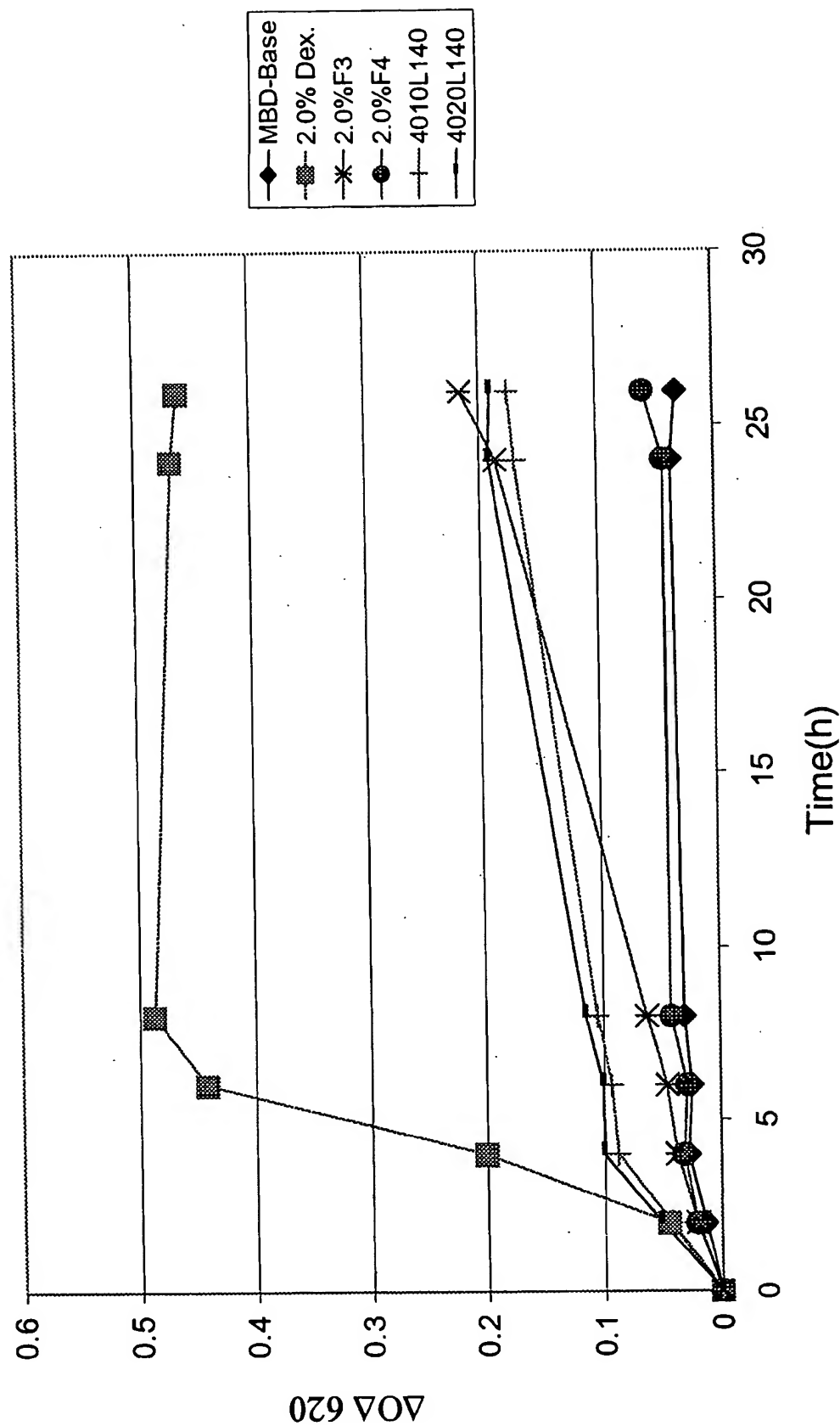


FIG. 26

FOS metabolism by *Salmonella* species group B(021901-5) using MBD media w & w/o sugars at 37 °C 5% CO<sub>2</sub> incubation 3/9/01.

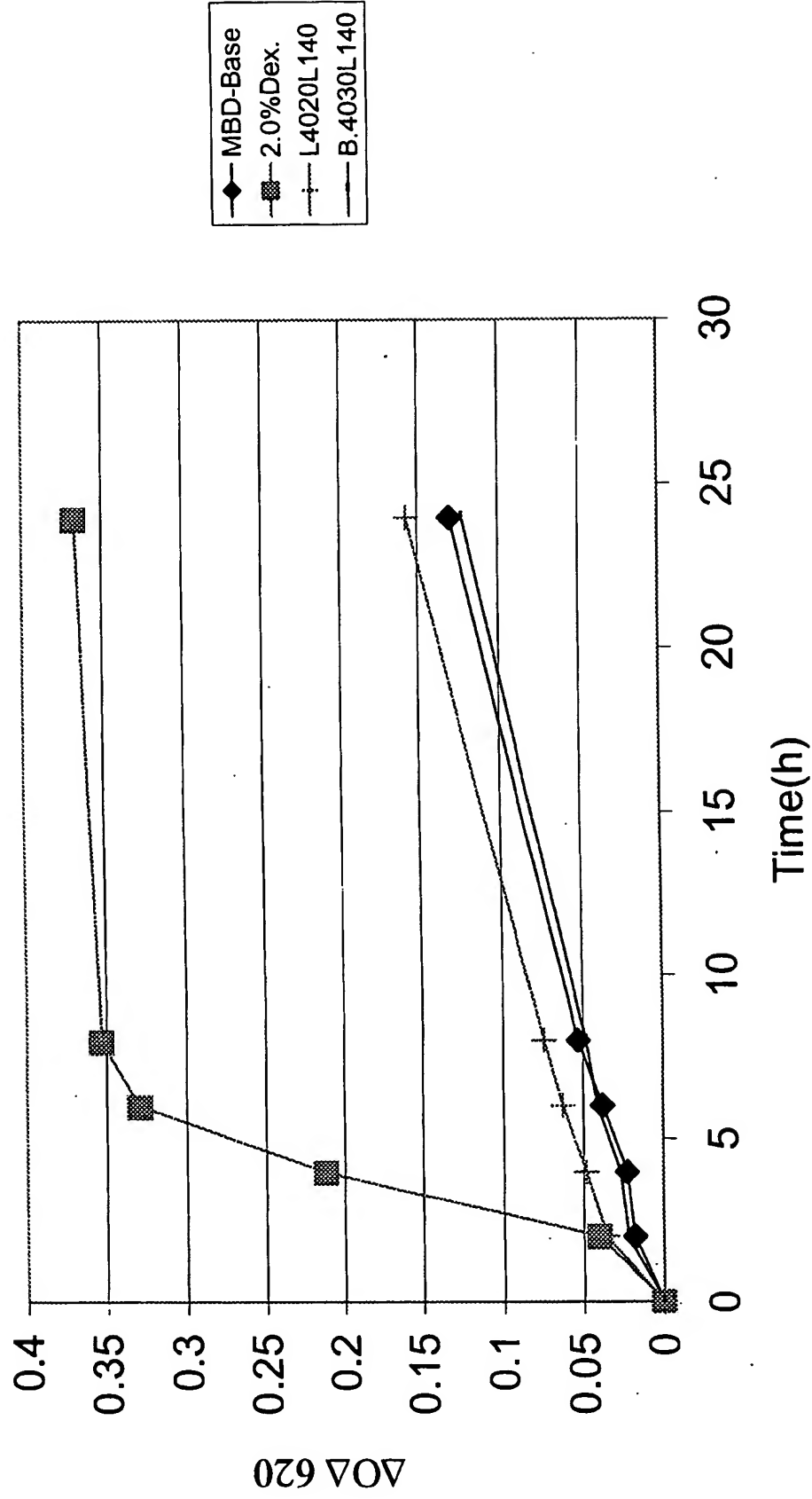


FIG. 27

# Cloned Levansucrases

Bacillus subtilis  
Gluconacetobacter diazotrophicus  
Bacillus stearothermophilus  
Pseudomonas aurantiaca  
Pseudomonas syringae pv. Glycinea  
Rahnella aquatilis  
Zymomonas mobilis  
Erwinia amylovora  
Paenibacillus polymyxa  
Acetobacter xylinus  
Bacillus amyloliquefaciens

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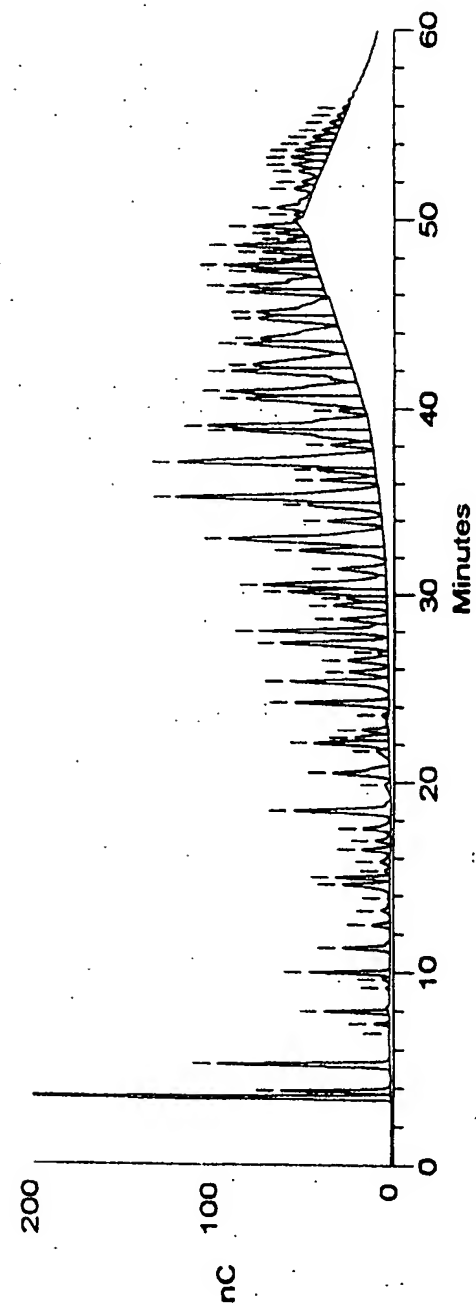
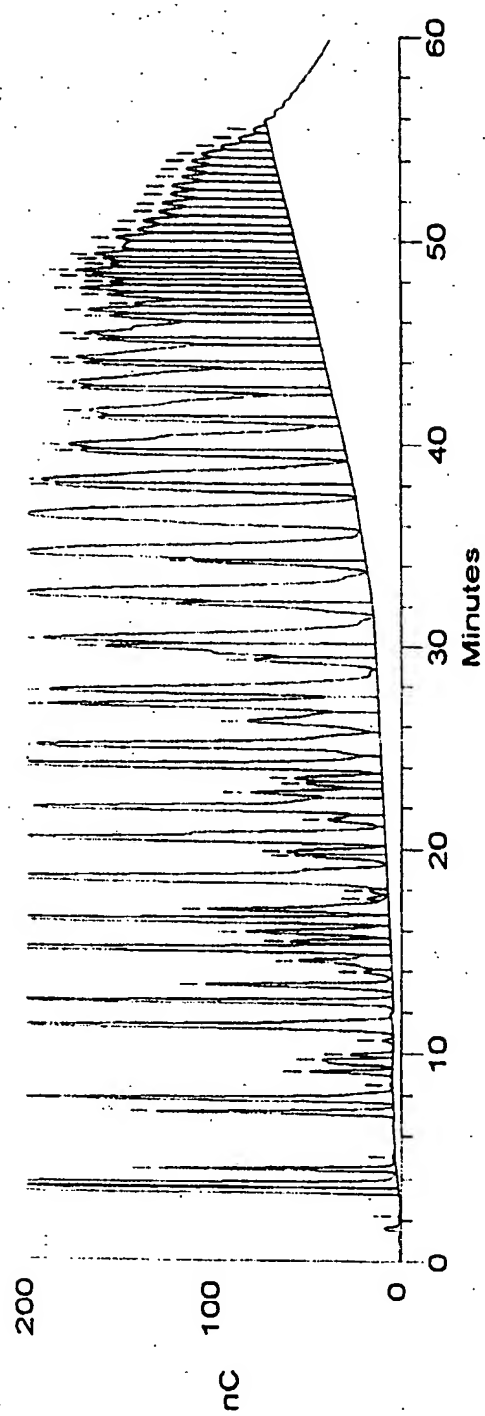


FIG. 29

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# Levan cGMP batch

Input: 62.88 kg sucrose

150 Units *B. subtilis* levansucrase

Reaction: 81.2 L @ 50°C X 65 hours

	kg levan	kg sucrose	kg glucose	kg fructose
100K filtrate	16.03	4.40	26.40	10.66
post- Amberlite	15.791	4.35	26.00	10.57
G10 retentate	15.99	0.245	0.00	0.00

FIG. 30

## MALDI-TOF-MS of 4010LI40

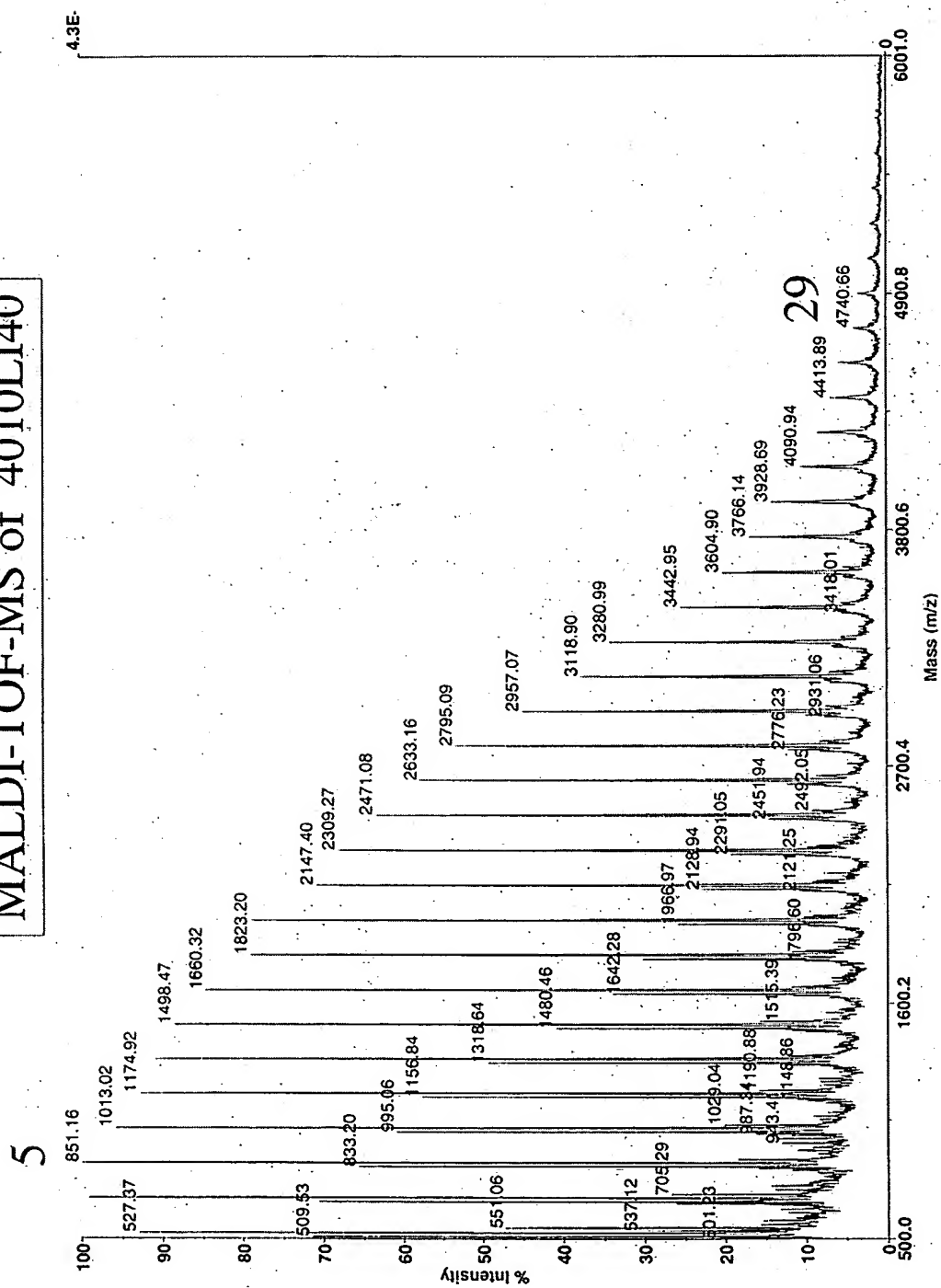


FIG. 31



# Size Exclusion / Light Scattering Analysis

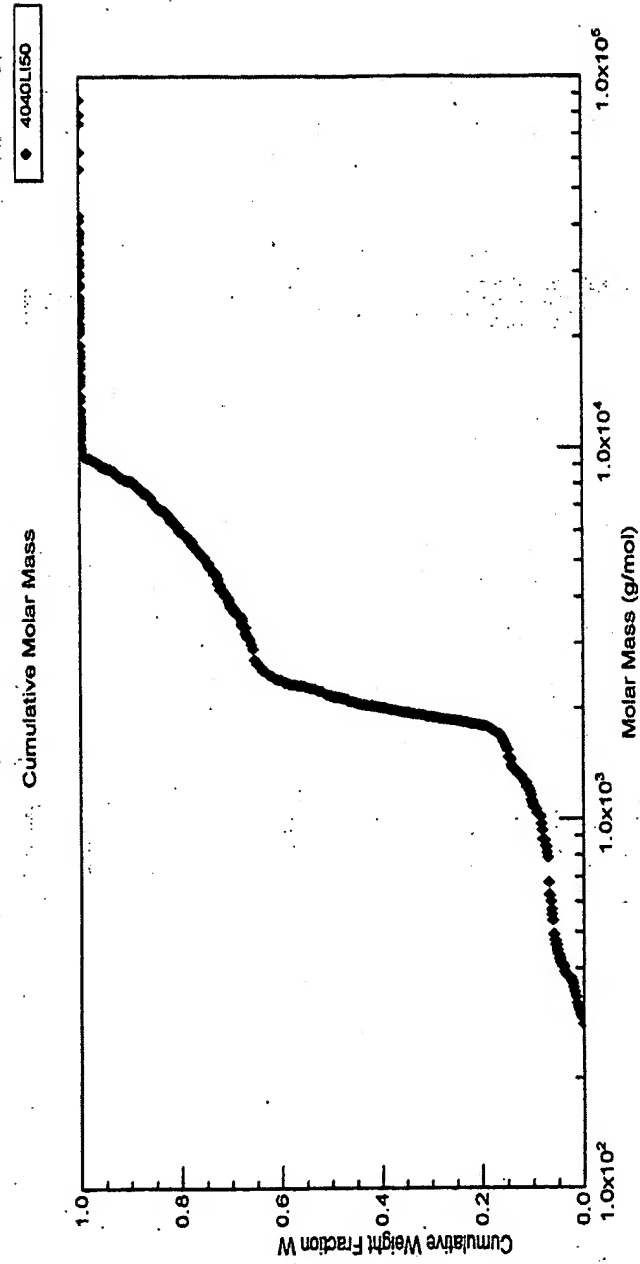


FIG. 32

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Baselines - 4040LI50

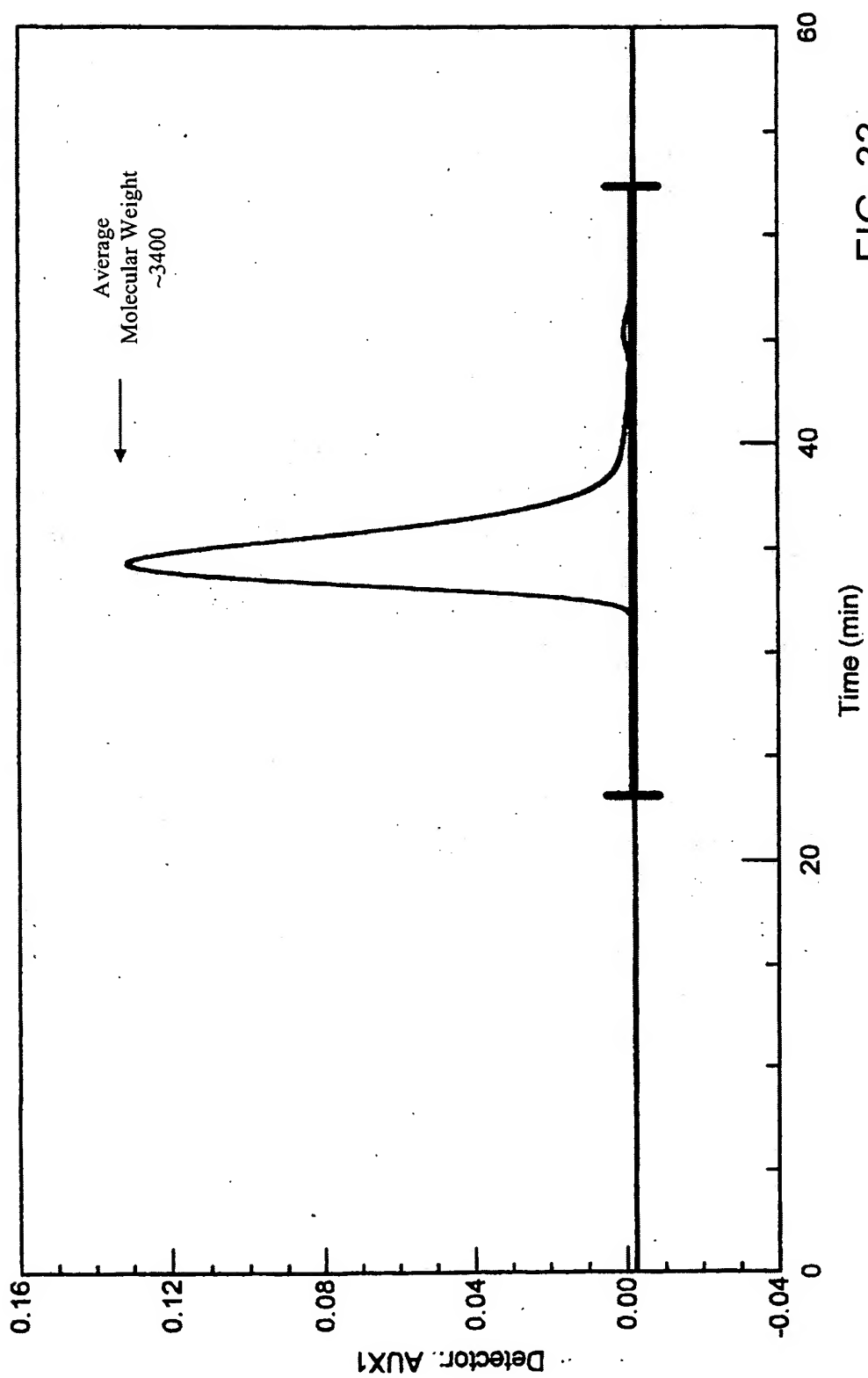


FIG. 33

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## Compound Comparisons

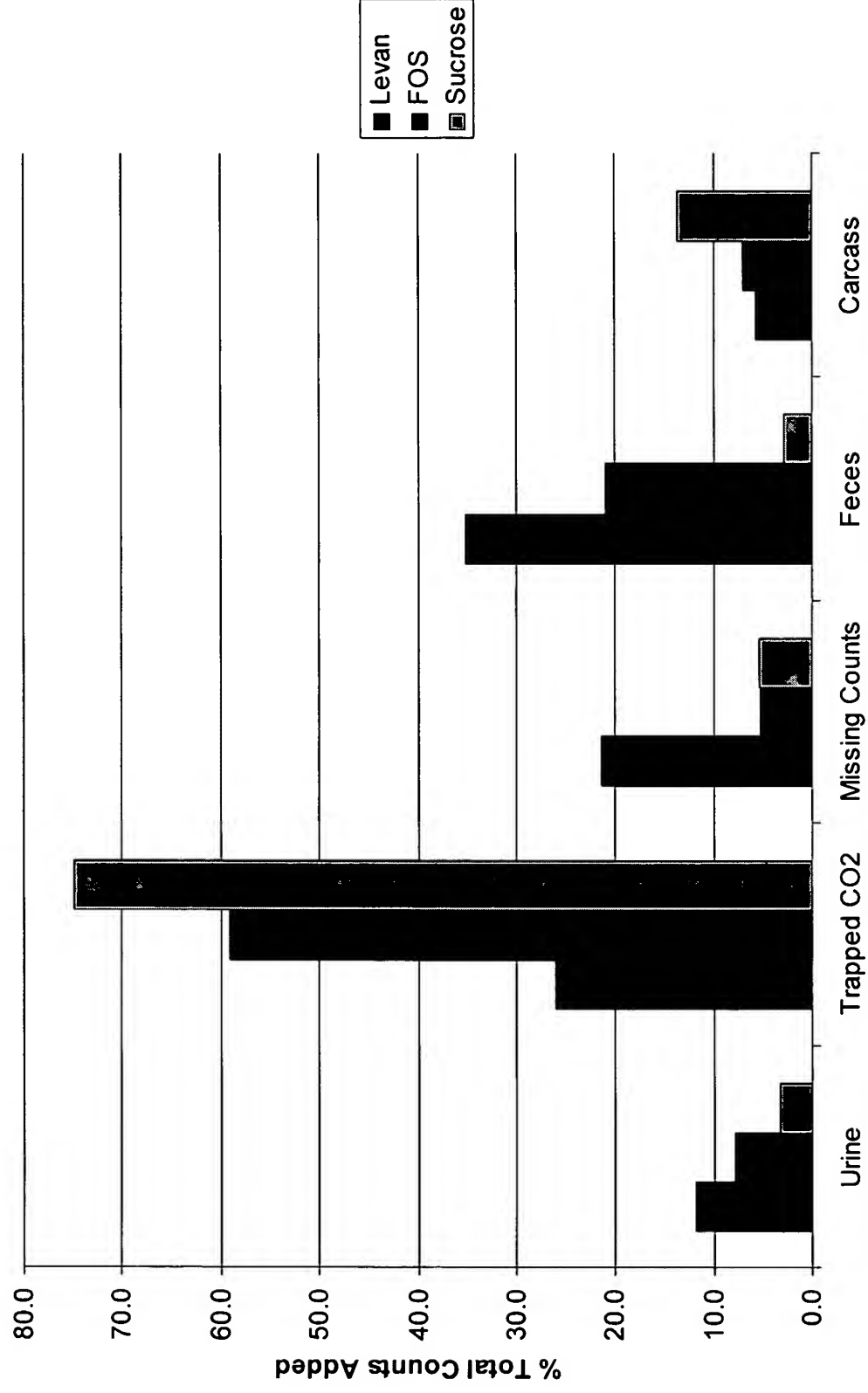


FIG. 34

# Trapped CO<sub>2</sub> - Time Course

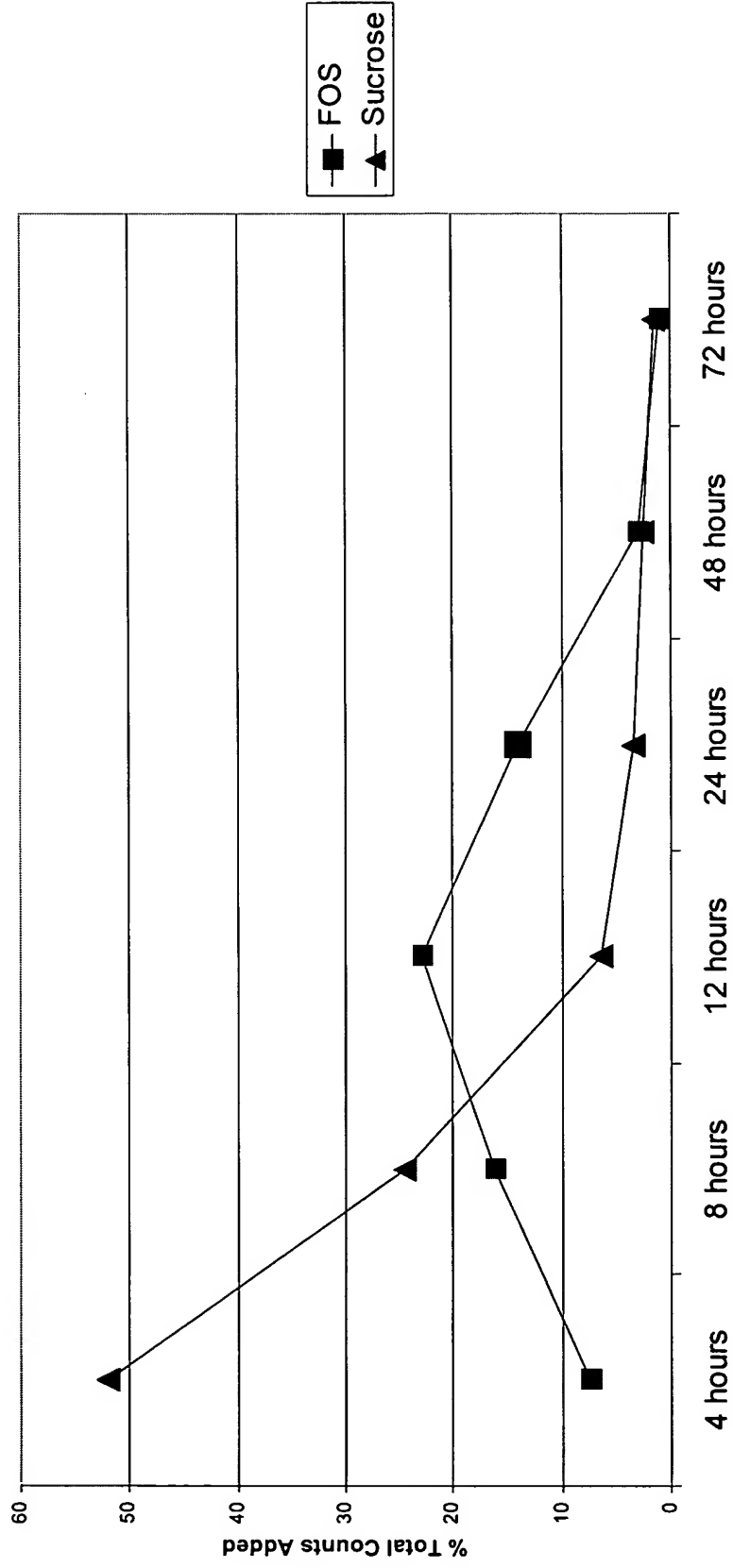


FIG. 35

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## Compound Comparisons

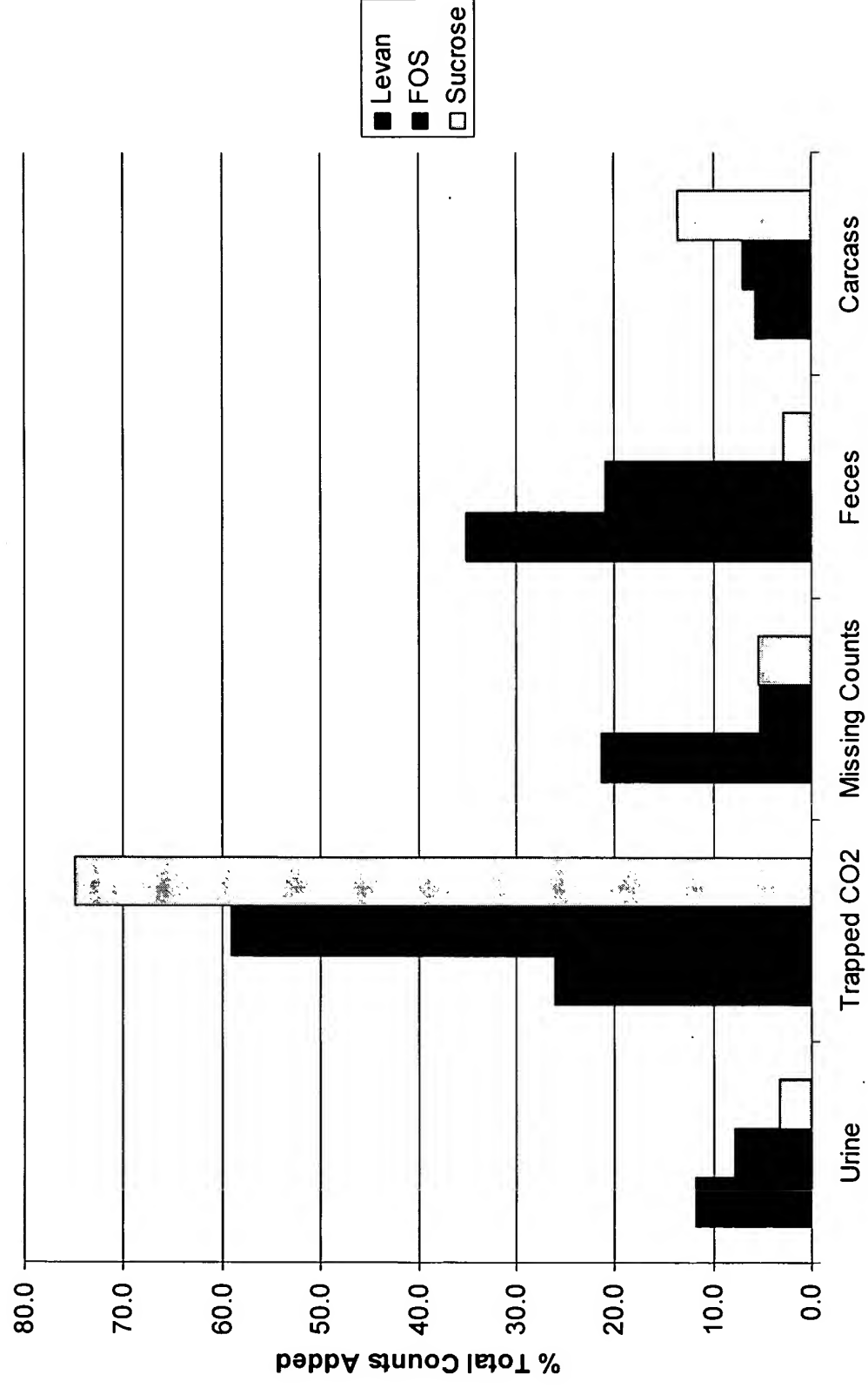


FIG. 36

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# Time Course - Trapped Gases

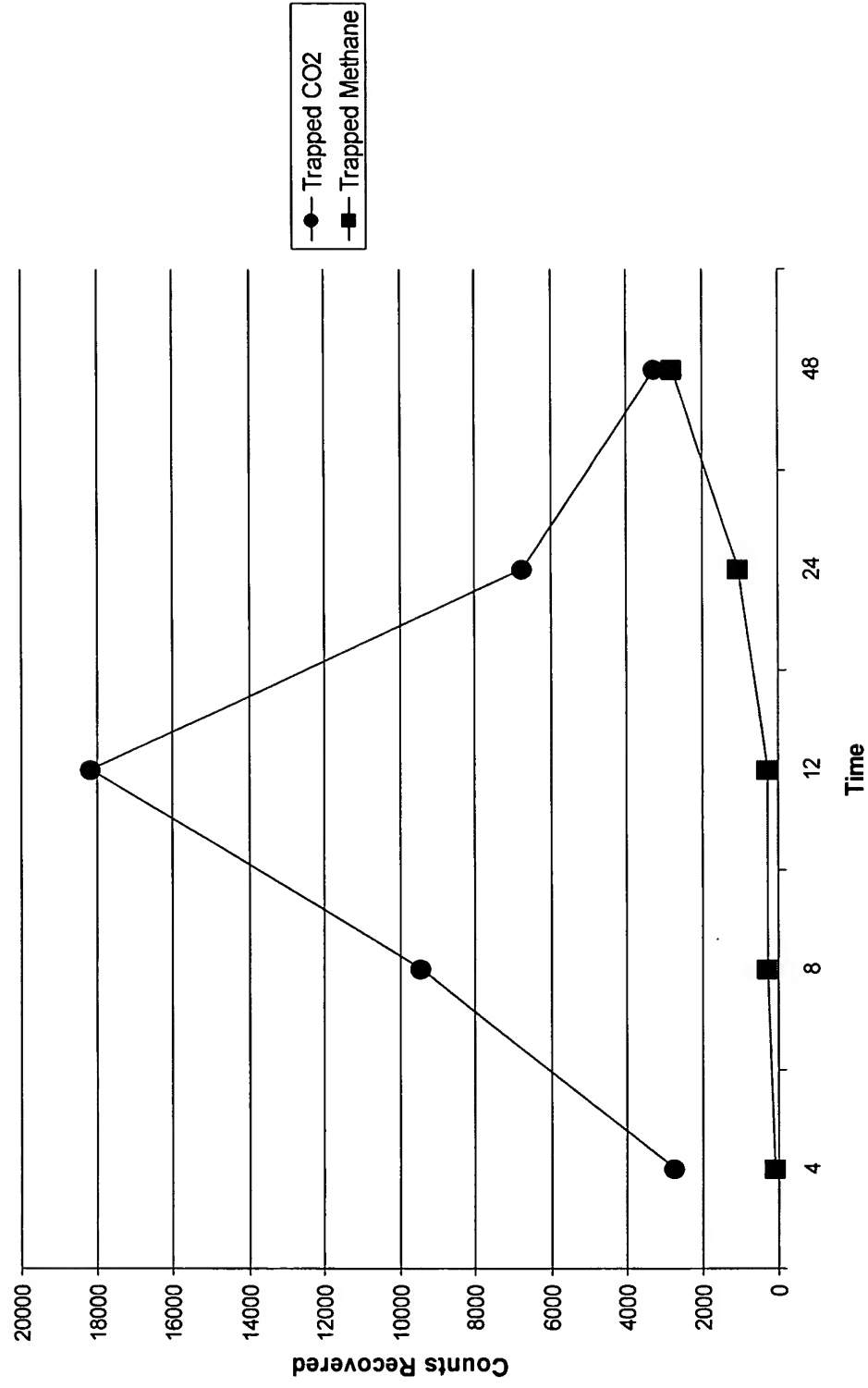


FIG. 37

## Branched fructan GMP batch

Input: 17.8 kg FOS, 9.3 kg sucrose (~2:1 ratio)  
15 Units *B. subtilis* levansucrase

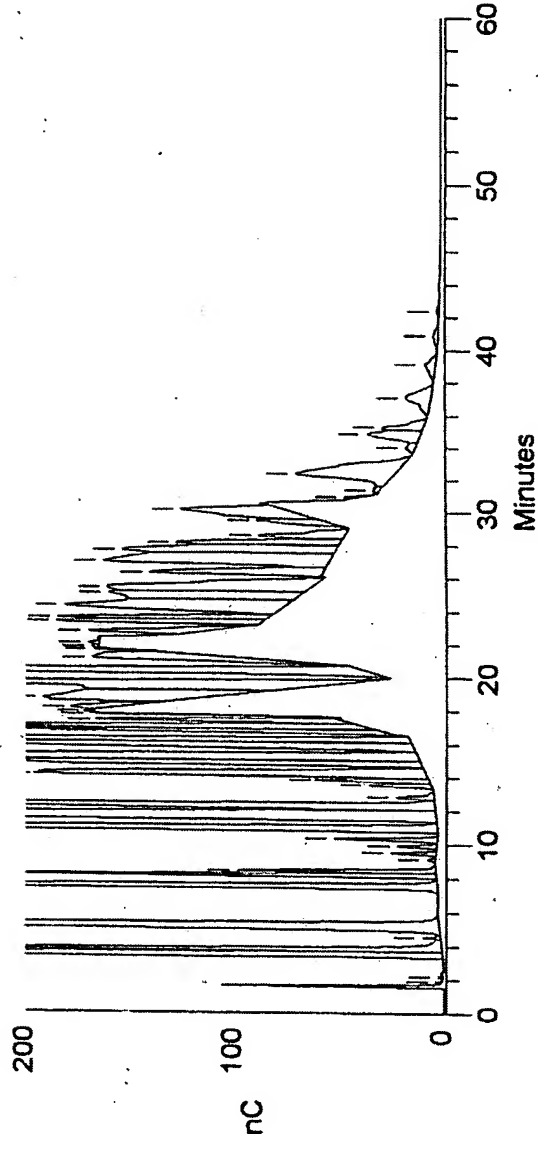
Reaction: 36.1 L @ 50°C X 24 hours

	kg br. fructan	kg sucrose	kg glucose
100K filtrate	20.84	0.43	4.74
Post-Amberlite	19.47	0.40	4.39
G10 retentate	18.39	0.13	0.21

FIG. 38

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Branched fructan @ 50°C  
2:1 FOS:sucrose



Branched fructan @ 35°C  
1:1 FOS:sucrose

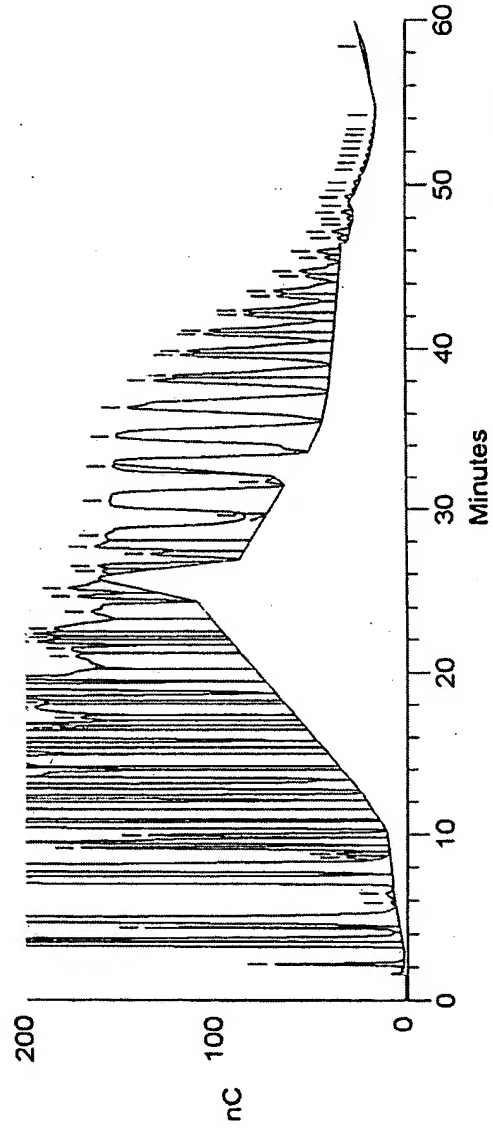
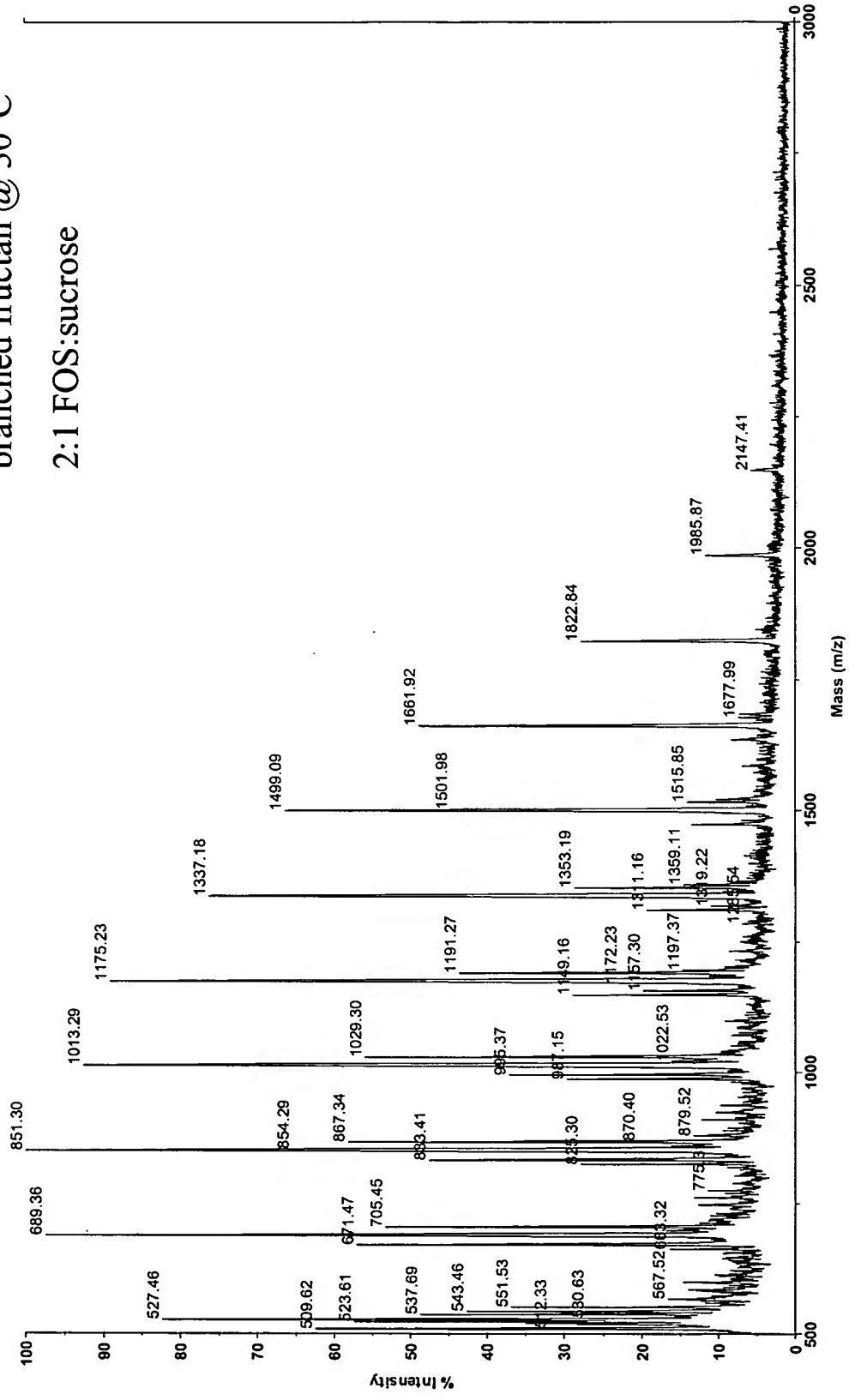


FIG. 39





## Branched Fructan Alternate Synthesis

Branched fructan can be synthesized from sucrose with two enzymes simultaneously.

Input: 4.6 kg sucrose

*A. niger* fructosyltransferase

*B. subtilis* levansucrase

(Total of 35 Units of both enzymes.)

Reaction: 6L @ 50°C X 16 hours

Reaction #1: 4:1 fructosyltransferase:levansucrase

Reaction #2: 1:1 fructosyltransferase:levansucrase

Reaction #3: 1:4 fructosyltransferase:levansucrase

## Branched fructan reaction #1

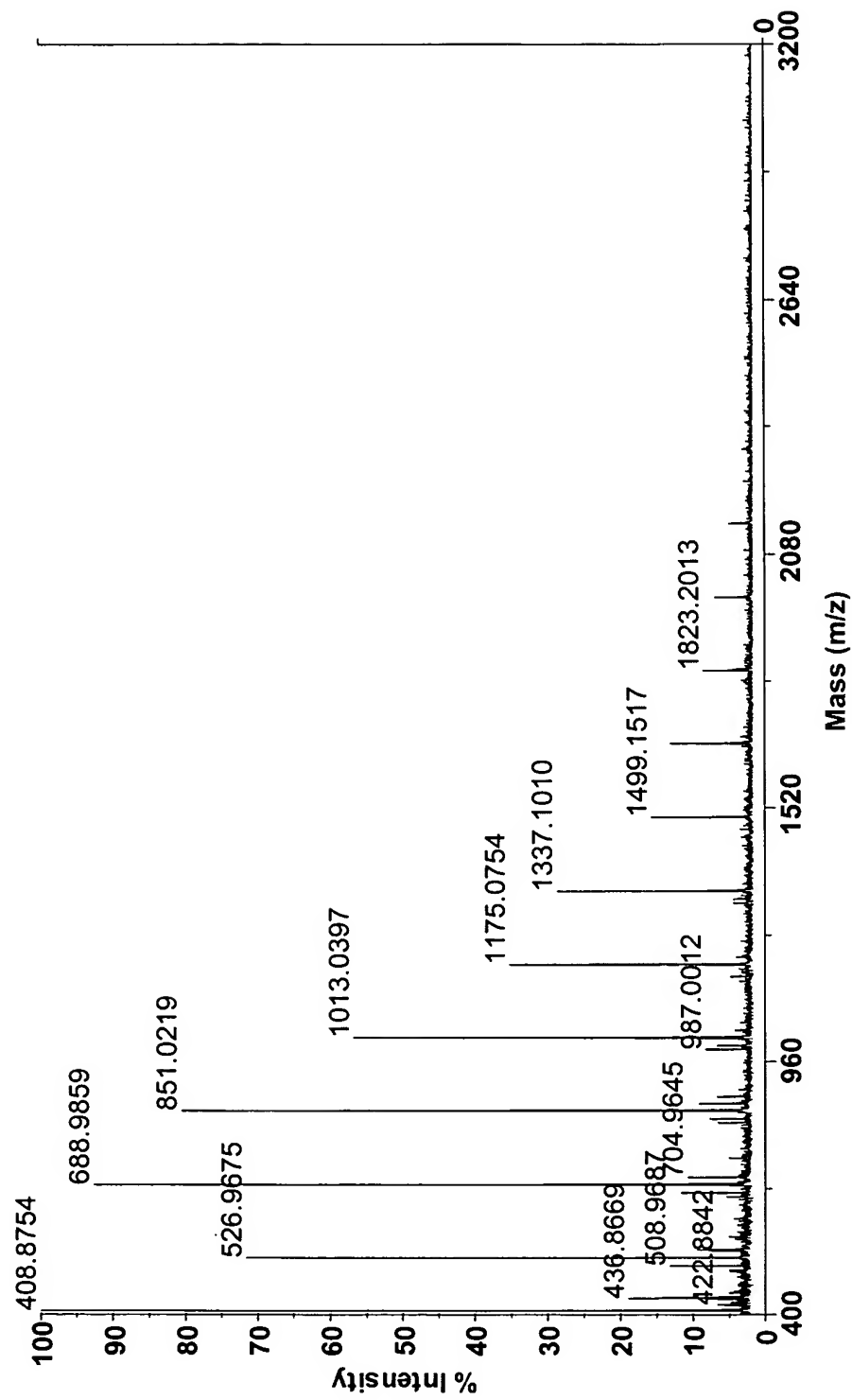


FIG. 42

## Branched fructan reaction #2

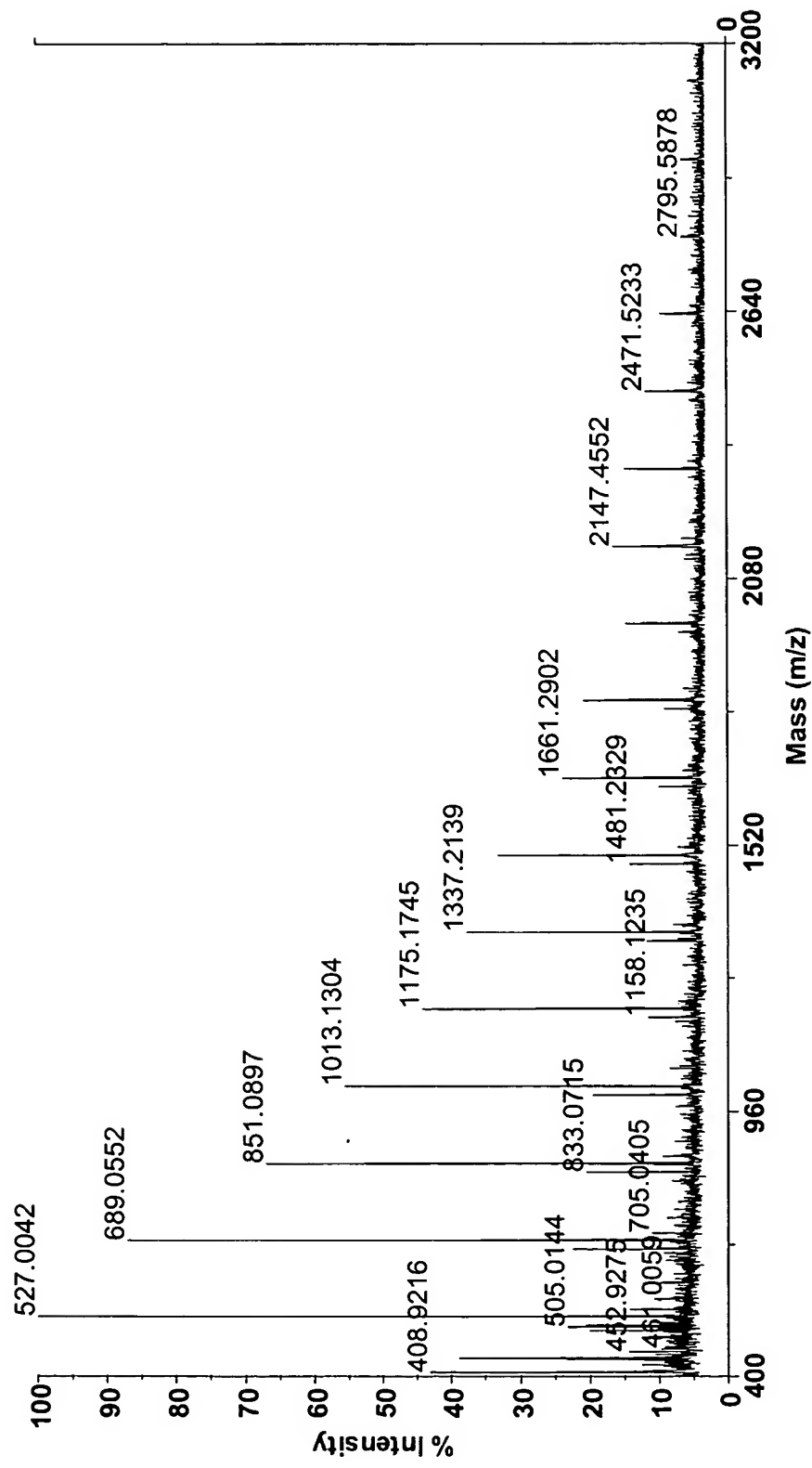


FIG. 43

## Branched fructan reaction #3

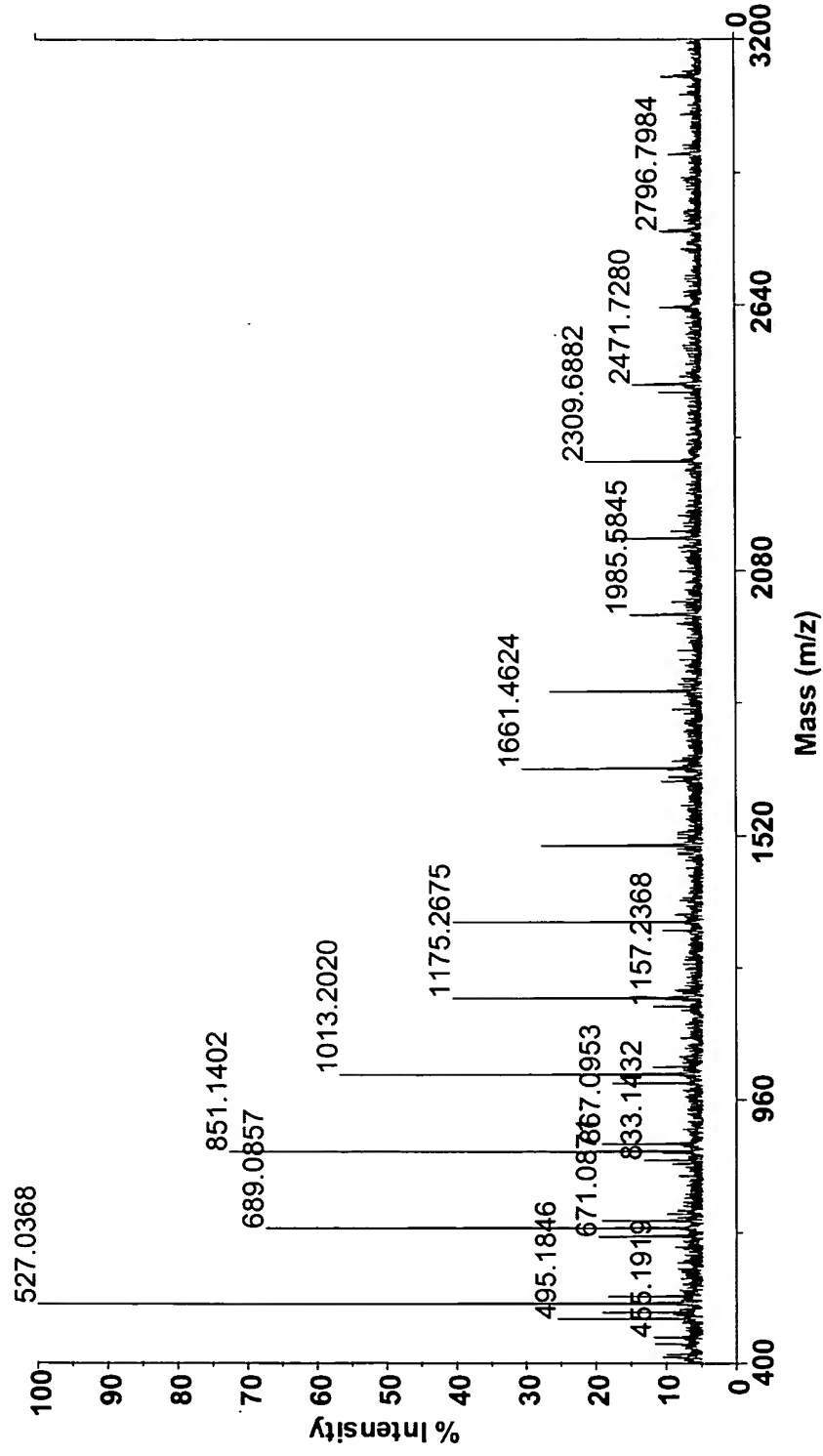


FIG. 44